THE INTERNATIONAL NETWORK ON SOIL FERTILITY AND FERTILIZER EVALUATION FOR RICE (INSFFER)

AN INTRODUCTION TO BLUE-GREEN ALGAE AND THEIR ROLE IN PADDY FIELDS

BY

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Blue-green algae (BGA) are photosynthetic prokaryotic microorganisms some of which are capable of nitrogen fixation. Such trophic independence with regard to nitrogen and carbon, together with a great adaptability to variations of environmental factors enables BGA to be ubiquitous.

The paddy field ecosystem provides an environment favorable for the growth of BGA with respect to their requirements for light, water, high temperature and nutrient availability. This may account for the higher abundance of BGA in paddy soils compared to other cultivated soils.

The agricultural importance of BGA in rice cultivation is directly related with the ability of certain forms to fix nitrogen. Contrary to heterotrophic N_2-fixing bacteria, BGA represent a self supporting system capable of both photosynthesis and N_2 fixation, the energy bill for both process being "paid by the sun".

Recent research, conducted mainly in India, has shown the feasibility of using BGA as a cheap additional nitrogen source for rice.

1 MORPHOLOGY AND TAXONOMY OF BGA

BGA have a wide range in form extending from simple unicells to multiseriate, true branching thalli. Due to the presence of various pigments (chlorophyll a, carotenes, xanthophylls, phycocyanin --

blue -- and phycoerythrin -- red) and mucilage, the color of BGA in nature may range from dirty yellow, through various shades of blue-green to brown or black.

11 CELLULAR ORGANIZATION

111 Vegetative cell

The BGA vegetative cell is of the prokaryotic type, lacking membrane - bounded DNA, golgi apparatus, mitochondria, plastids and chloroplasts.

The central area of the cell (centroplasm) contains granules of nuclear material. The peripheral plasm (chloroplasm) is usually more densely pigmented than the centroplasm and contains pigments which are concentrated in phycobilisomes located on the surface of photosynthetic lamella (thylakoids). Inclusions are mainly gas vacuoles, polyphosphate bodies, cyanophycin granules and lipid droplets - (cf Fig. 1).

112 Spores

Spores or akinetes are produced in heterocystous BGA, mainly in members of the Nostocaceae and Rivulariaceae. They are particularly resistant to adverse conditions and remain viable for long periods. They are distinguished readily under the light microscope by their large size, characteristic shape, modified pigmentation and the presence of many large cytoplasmic granules.

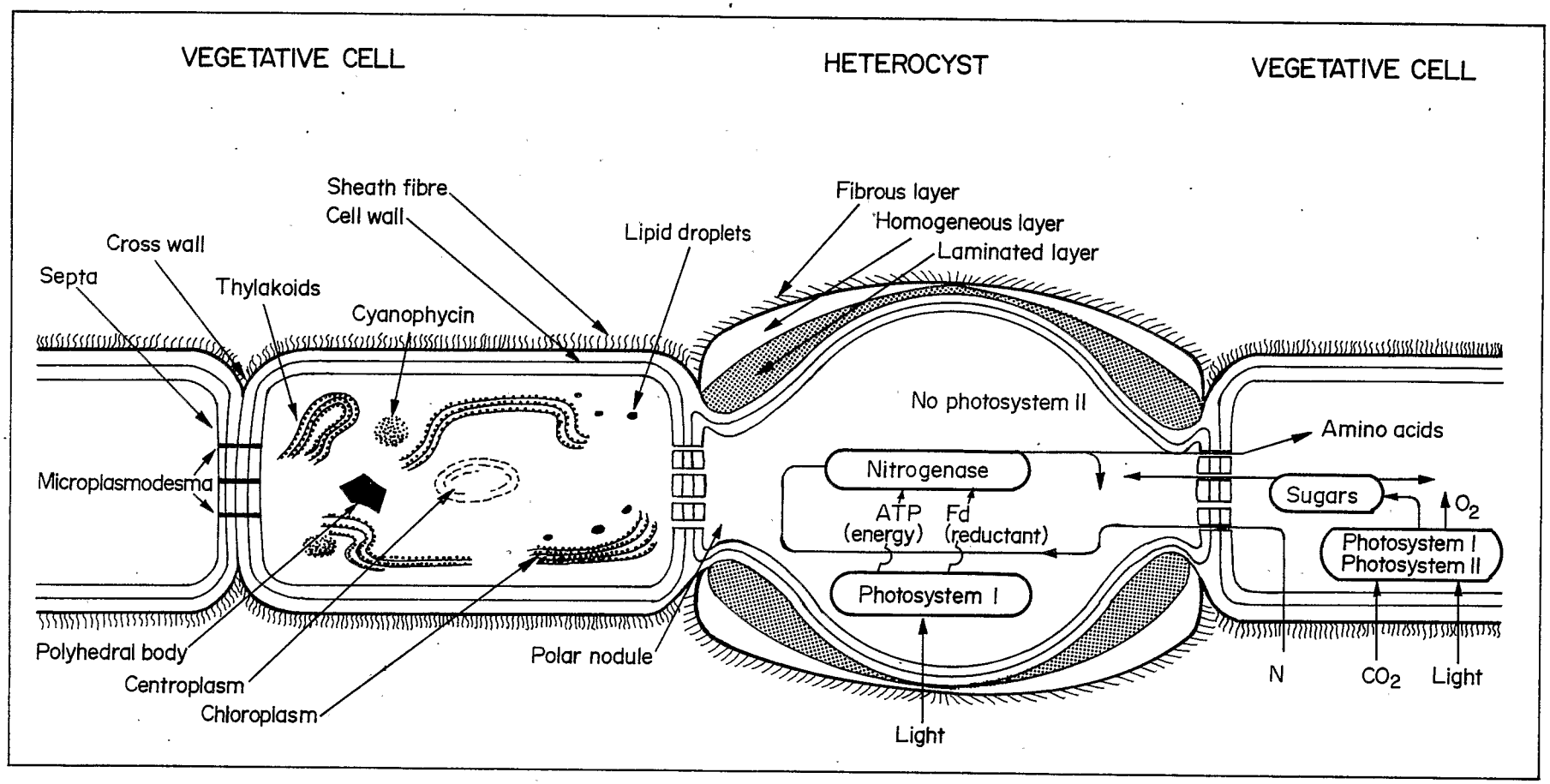


Fig. 1 Schematic representation of a vegetative cell, an heterocyst and physiological relationship between the two kind of cells.

113 Heterocysts

The heterocysts is a thick-walled, usually translucent, cell of certain BGA (heterocystous BGA) known to be the site of nitrogen fixation.

Heterocysts are produced from vegetative cells under nitrogen-limiting conditions. They occur either intercallary or basally. Heterocysts are readily recognized under the light microscope. Their main morphological features are:

- Round, squared or rectangular shape with a relatively large size as compared with the vegetative cells.
- Massive cellular envelope.
- Reduced pigmentation as compared with vegetative cells.
- Relatively homogeneous cellular content.
- Presence of polar nodules at their attachments to the vegetative cells.
- Generally produced in a specific spacing pattern along the filament.

114 Other structures

Other structures are either reproductive, like hormogonia and hormospores or vegetative like hairs, necridium, calyptra etc. A definition of these terms is given in a glossary, at the end of this handout.

12 MORPHOLOGICAL FEATURES AND TAXONOMY OF BGA

So far no method of sexual reproduction or the presence of any sexual reproductive units has been demonstrated among these

organisms. Their classification is therefore based almost entirely upon morphological features. The more important ones are as follows:

a. growth form - unicellular, colonial, filamentous

b. Compactness and shape of colony

c. among the filamentous members

i) branching - false and true

ii) cell differentiation - heterocysts and akinetes

iii) polarity - base and apex of filament distinguishable

iv) sheath - absence/presence; thickness

v) nature of false branching - single (non-geminate), double (geminate)

vi) nature of true branches: uniseriate, multiseriate

vii) size and shape of cells, heterocysts and akinetes; principally for the identification of species.

A simplified schematic representation and drawings of the main genera are provided herewith to illustrate the diversity of form among blue-green algae, together with two examples of classifications and the corresponding keys.

The classification of DESIKACHARY, simplified by VENKATARAMAN is an example of a "classical" classification which permits the determination of the genera and species of fresh samples but which is frequently useless for strains growing on artificial media like culture collection or colonies growing on Petri dishes after inoculation with suspension dilution of soil.

The classification of RIPPKA et al was designed especially for culture collection strains but does not permit the determination of the species.

Table 1

Schematic representation of the morphological diversity among blue-green algae.

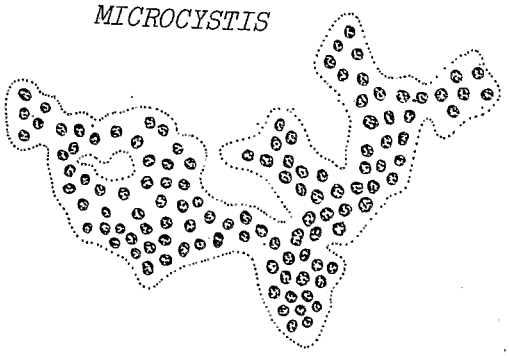
- BGA
  - Unicellular
    - Simple unicells: *Anacystis*
    - Cell aggregates of irregular shape
      - Loose colonies: *Microcystis, Aphanothece*
      - Compact colonies: *Gloeocapsa*[a]
    - Cell aggregates of definite shape
      - Flat plates: *Merismopedia*
      - Cuboidal colonies: *Eucapsis*
  - Filamentous
    - Without true branching
      - Without false branching
        - Without cell differentiation: *Oscillatoria* (thin sheath), *Lyngbya* (thick sheath), *Spirulina* (spiral filament), *Microcoleus* (several trichomes within one sheath)
        - With cell differentiation: *Nostoc*[a], *Anabaena*[a], *Cylindrospermum*[a]
      - With false branching
        - Filament with polarity (tapering)
          - Noncolonial: *Calothrix*[a]
          - Colonial: *Rivularia* (non-sporing)[a], *Gloeotrichia* (sporing)[a]
        - No polarity (non-tapering)
          - Without cell differentiation: *Plectonema*
          - With cell differentiation: *Scytonema*[a] (geminate branches), *Tolypothrix*[a] (single branches)
    - With true branching
      - Nonheterotrichous thalli
      - Heterotrichous thalli (primary branches prostrate, secondary branches erect)
        - Both primary and secondary branches uniseriate: *Mastigocladus*[a], *Hapalosiphon*[a]
        - Primary branches uniseriate secondary branches multiseriate: *Fischerella*[a]
        - Both primary and secondary branches multiseriate: *Stigonema*[a]

[a]Fix nitrogen in air.

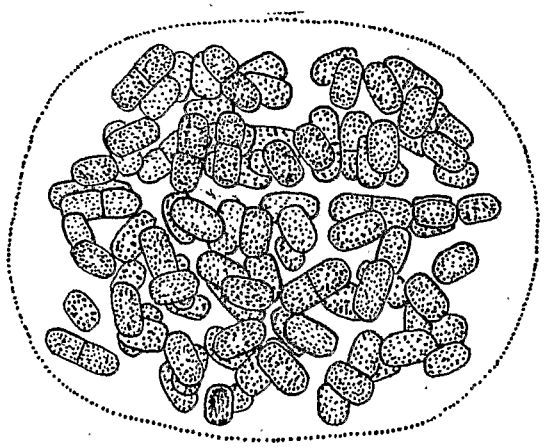
PLATE I

FREE AND COLONIAL UNICELLULAR FORMS

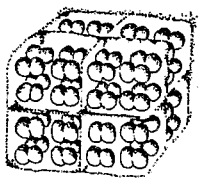
MICROCYSTIS



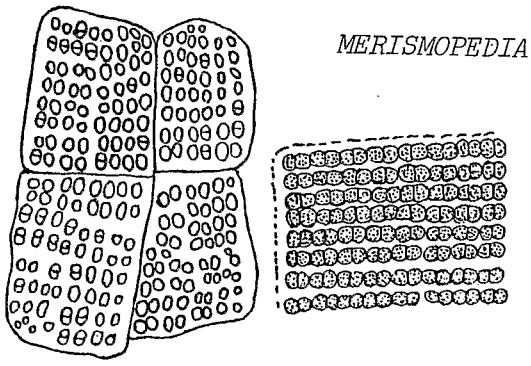
APHANOTHECE



EUCAPSIS



MERISMOPEDIA



GLOEOCAPSA

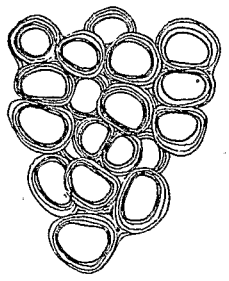
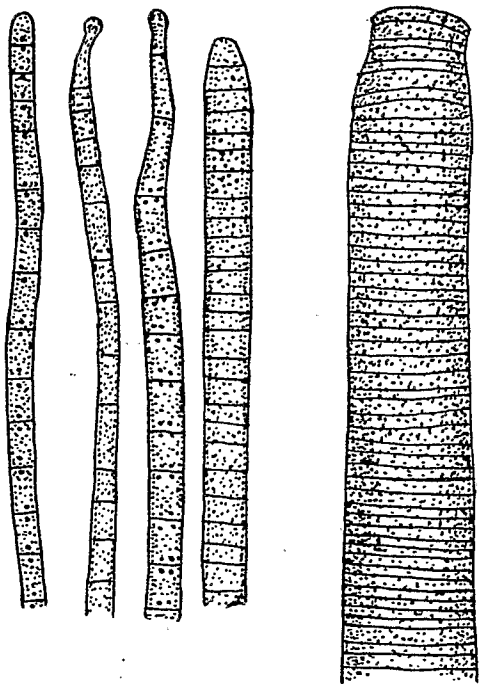


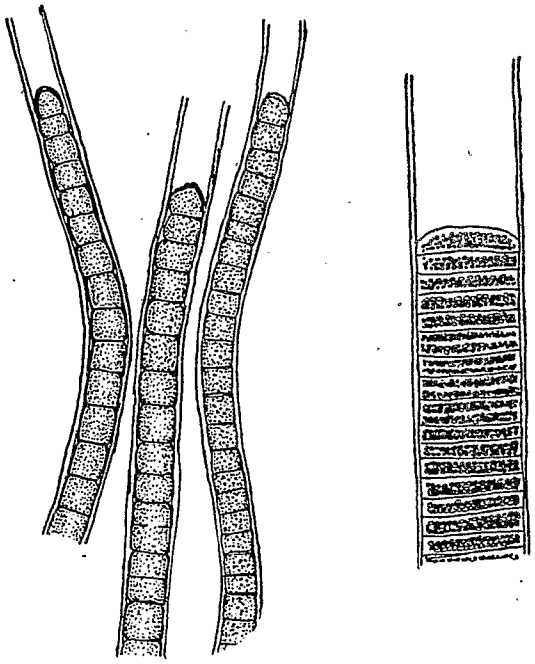
PLATE 2

FILAMENTOUS FORMS WITHOUT BRANCHING AND WITHOUT CELL DIFFERENTIATION

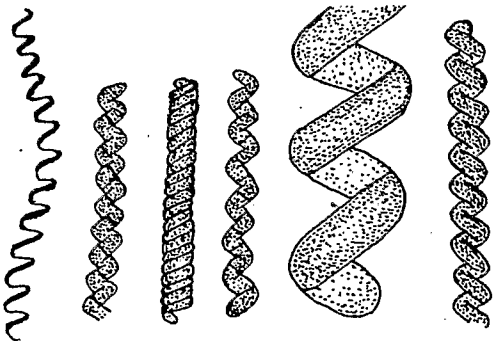
OSCILLATORIA



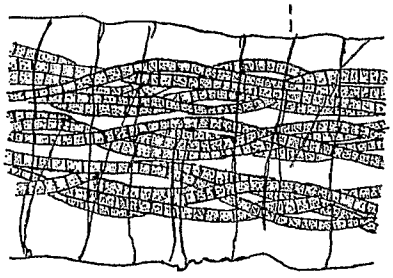
LYNGBYA



SPIRULINA

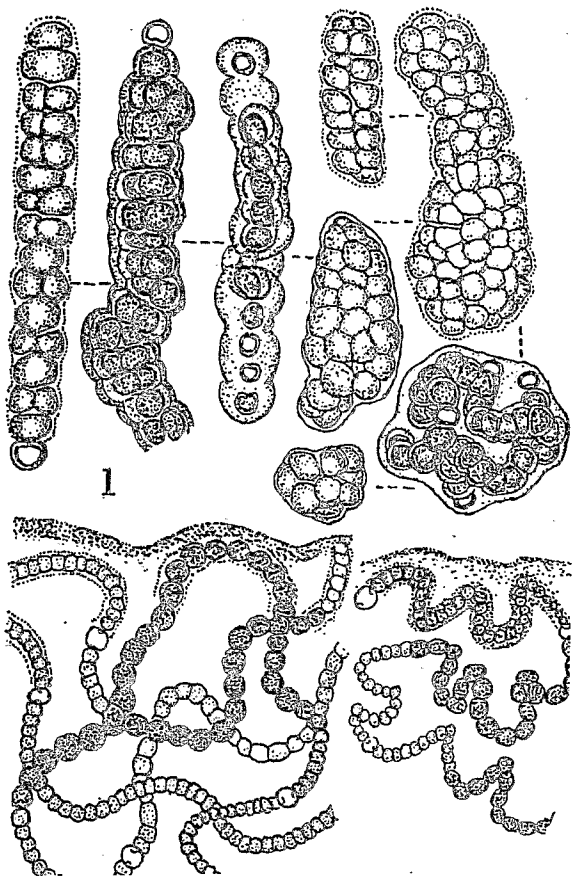


MICROCOLEUS

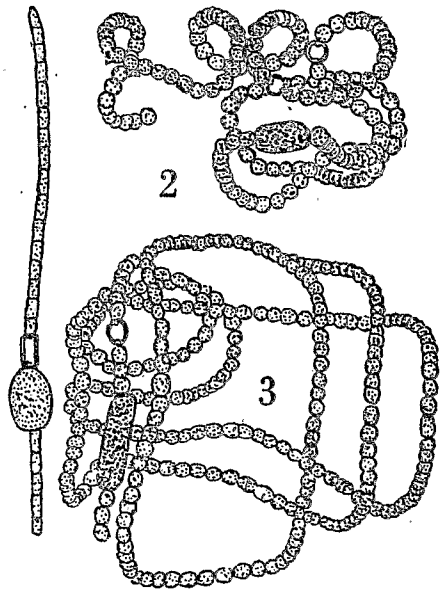


FILAMENTOUS FORMS WITHOUT BRANCHING AND WITH CELL DIFFERENTIATION

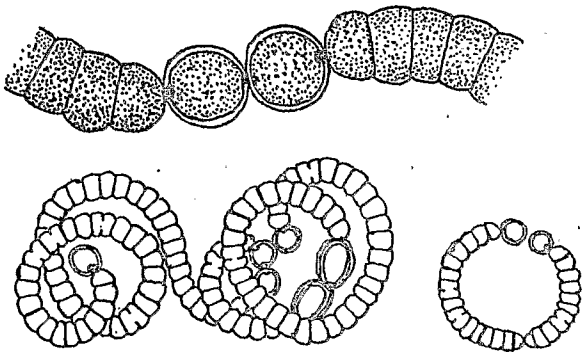
NOSTOC



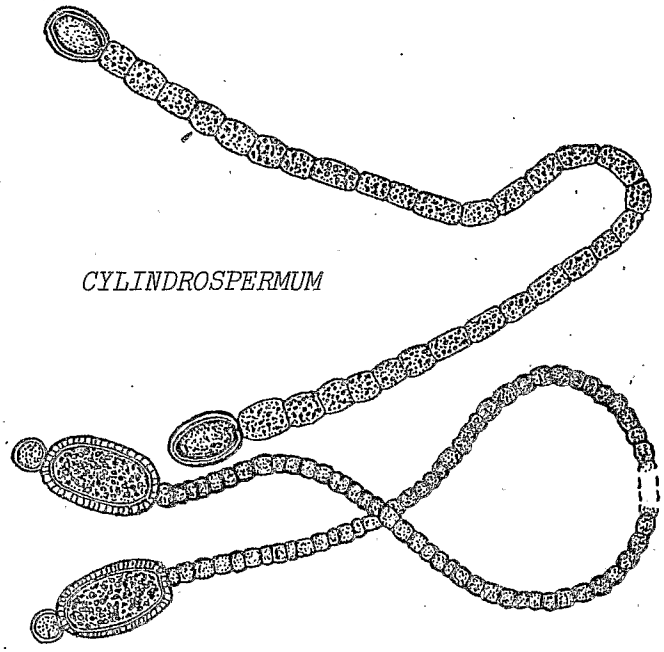
ANABAENA



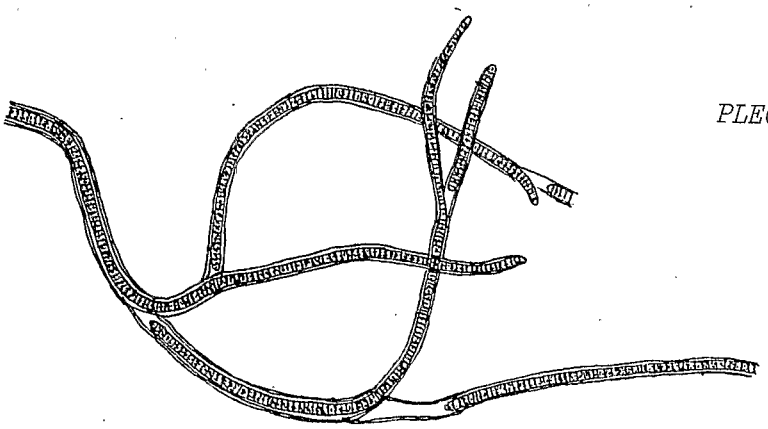
ANABAENOPSIS



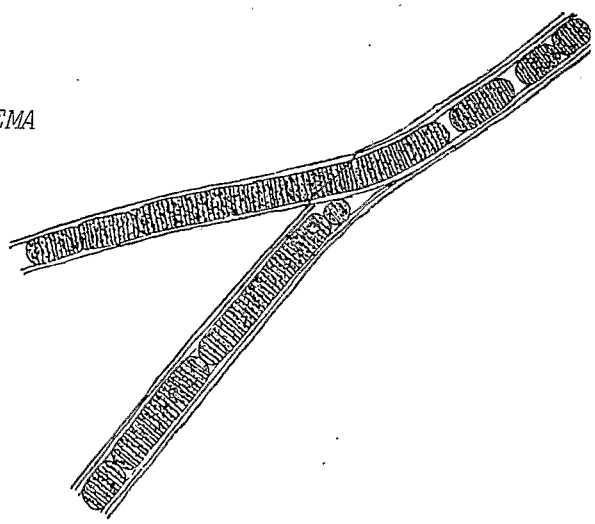
CYLINDROSPERMUM

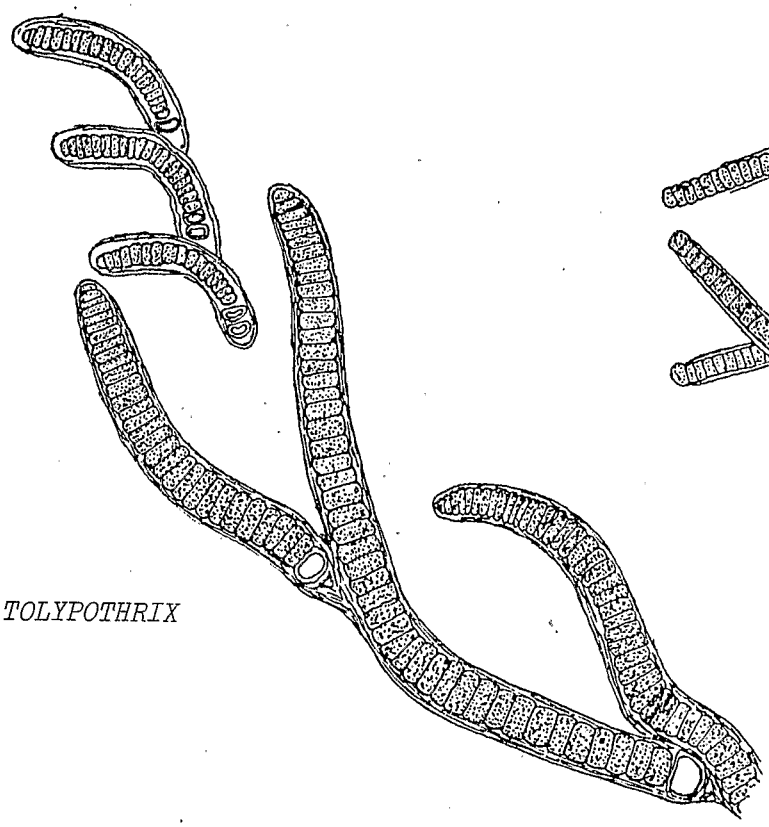


FILAMENTOUS FORMS WITHOUT TRUE BRANCHING, WITH FALSE BRANCHING, AND WITHOUT POLARITY



PLECTONEMA





TOLYPOTHRIX

SCYTONEMA

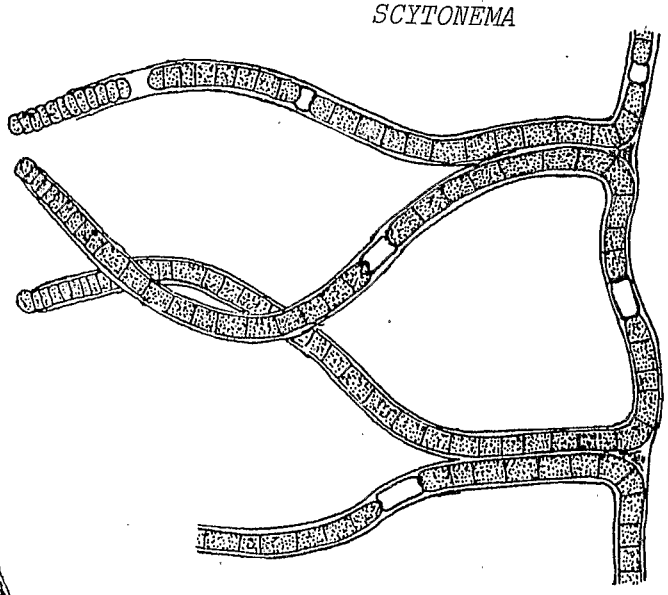
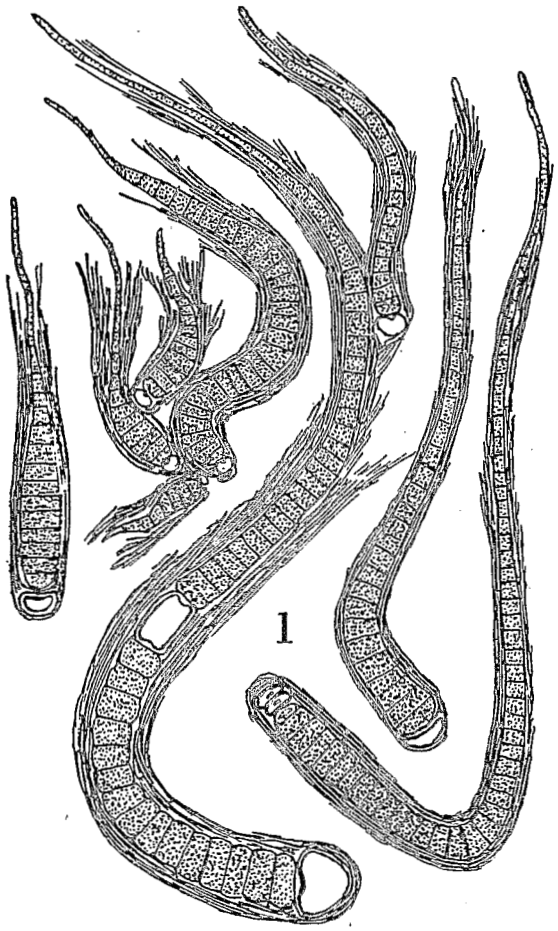


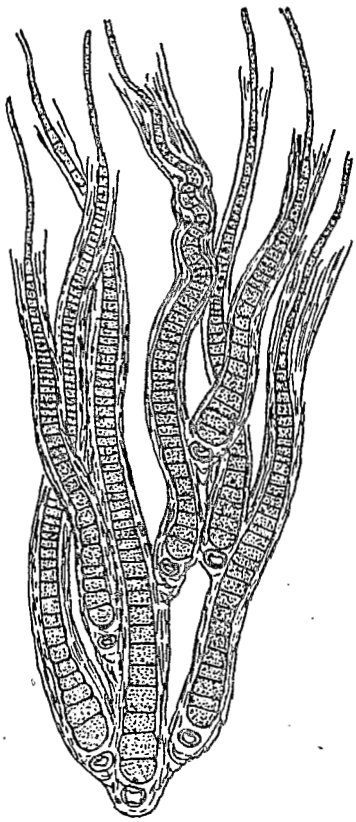
PLATE 5

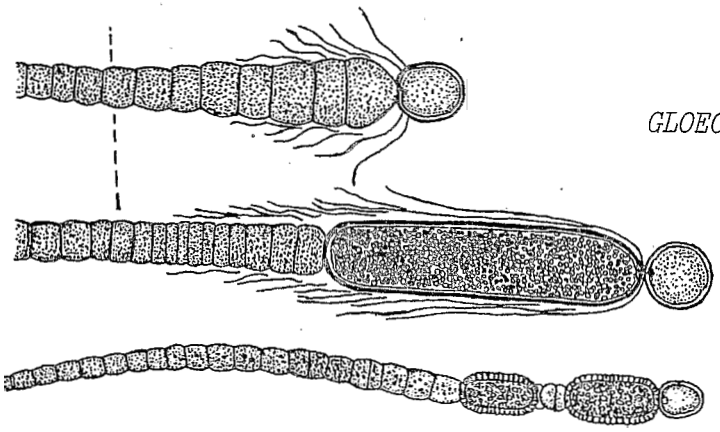
FILAMENTOUS FORMS WITHOUT TRUE BRANCHING, WITH FALSE BRANCHING, AND POLARITY

CALOTHRIX



RIVULARIA





GLOEOTRICHIA

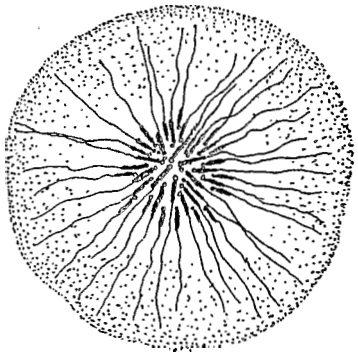
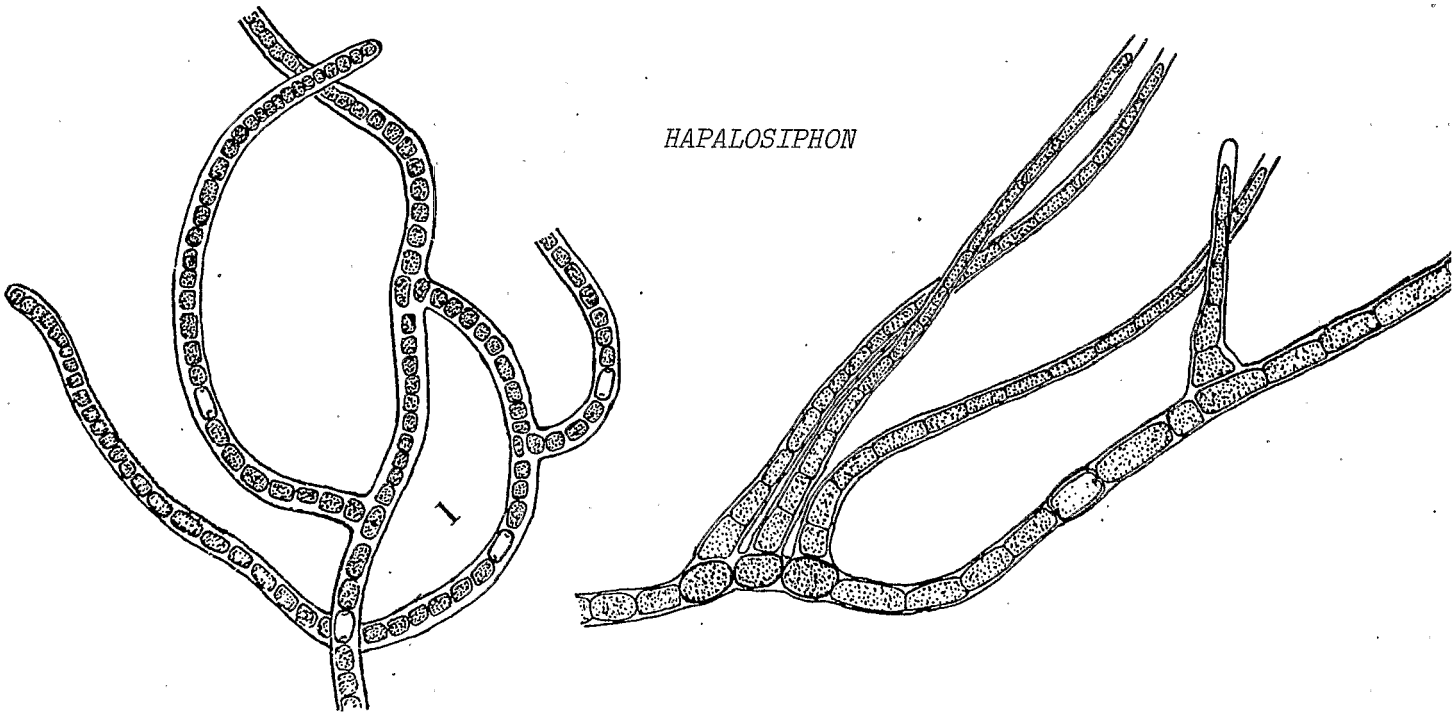


PLATE 6

FILAMENTOUS FORMS WITH TRUE BRANCHING UNISERIATE



MASTIGOCLADUS

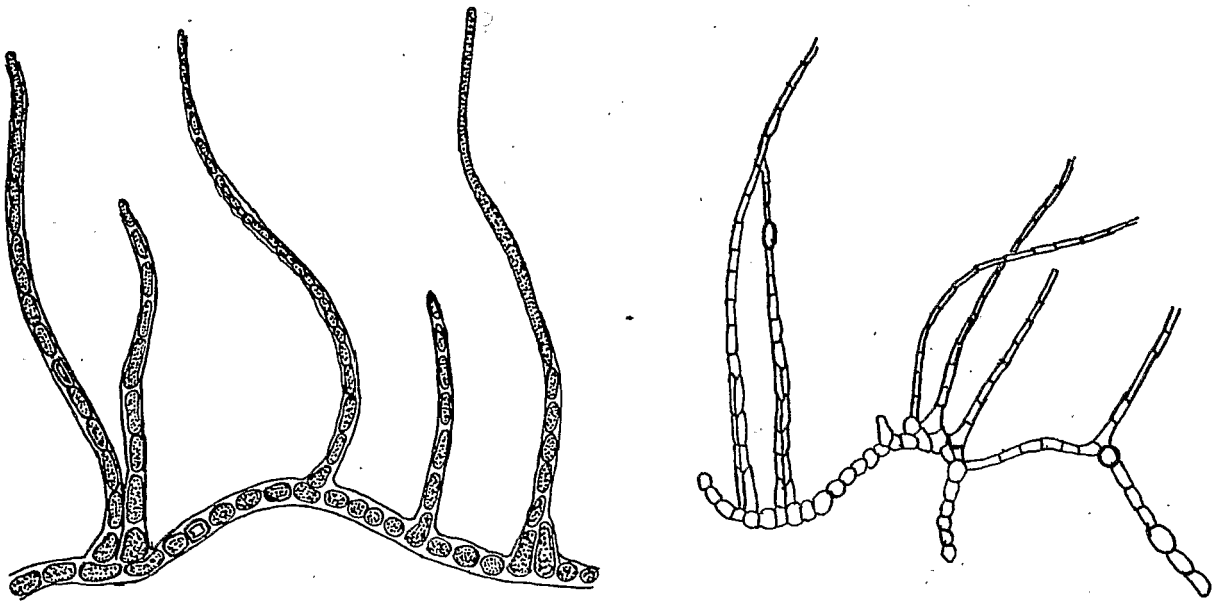
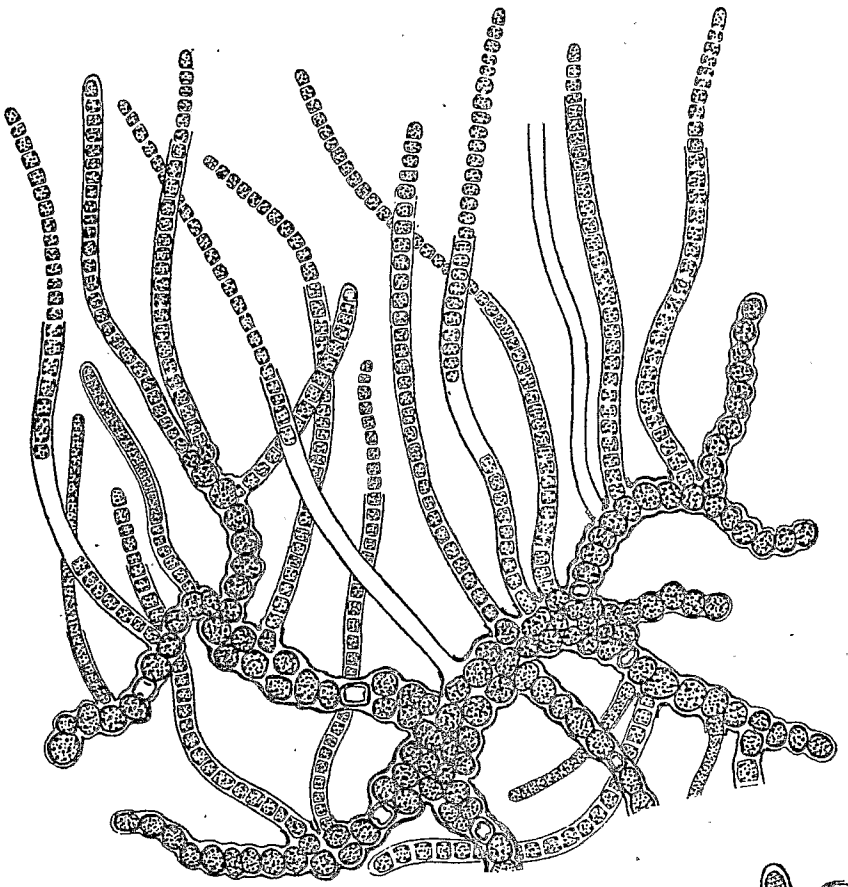
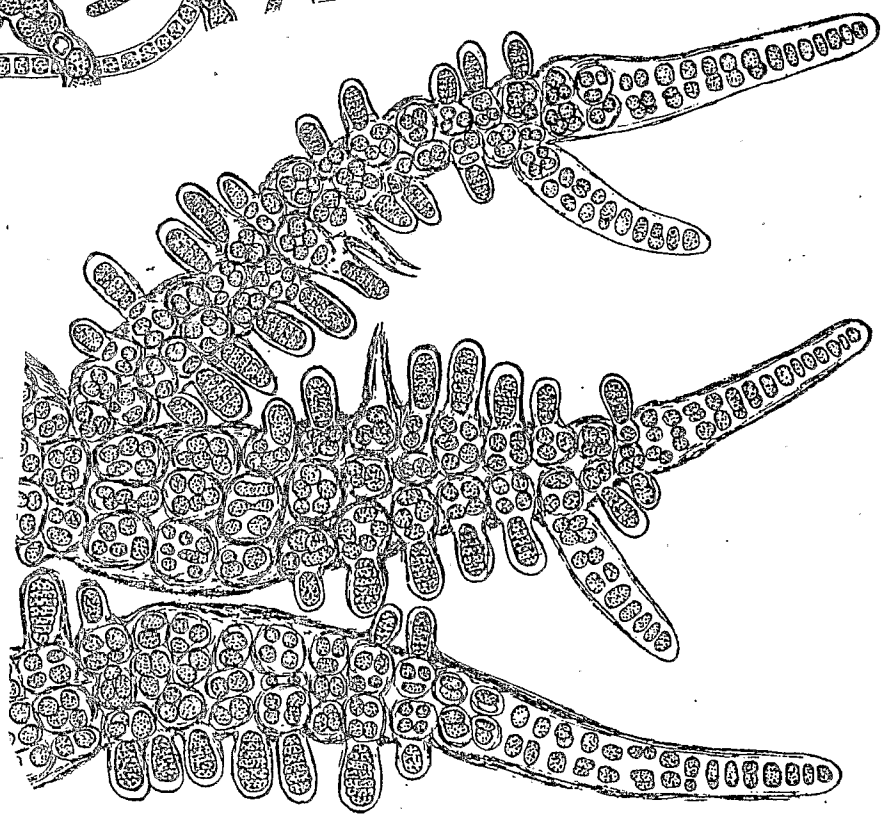


PLATE 7

FILAMENTOUS FORMS WITH TRUE BRANCHING, MULTISERIATE



FISCHERELLA



STIGONEMA

THE CLASSIFICATION OF BGA BY DESIKACHARY, SIMPLIFIED BY VENKATARAMAN
(Reproduced from Blue-green algae for rice production - FAO Soils Bulletin No. 46. 1981 by G. S. Venkataraman).

Following keys do not cover all the families and genera of BGA. They have been selected by G. S. Venkataraman (1969) in order to help in identifying the forms for which there is evidence of N_2-fixing capacity. For complete keys, the reader is referred to DESIKACHARY (1969).

KEY TO THE ORDERS

Plants unicellular or colonial, sometimes forming a pseudofilamentous colony, never with a 'trichome organization', no differentiation into base and apex, endospores not formed in sporangis; no exospores; nannocytes present.

Chroococcales

Plants unicellular, attached, typically differentiated into base and apex, reproduction by endospores or exospores.

Chmaesiphonales

Plants more or less distinctly filamentous, attached, arrangement very uniform, chrococcaceous structure, often forming parenchymatous thalli with prostrate and erect filaments, without differentiation into trichome and filament, no hormogones, no heterocysts, endospores in sporangia.

Plerocapsales

Plants filamentous with trichome and filament organized or 'hormogonalen organization', hormogones present, often with heterocysts, akinetes, exospores or endospores, pseudohormogonia present.

Without true branching, unbranched, or with false branching.

Nostocales

With true branching or dichotomous branching and often with heterotrichous condition, i.e. with a differentiation of prostrate and erect portions.

Stigonematales

KEY TO THE GENERA

ORDER CHROOCOCCALES

Cells unicellular or forming colonies, not forming filamentous growth; cells generally many in a single colony; cells without any regular or definite arrangement, cells with distinct individual envelopes or sheath; colonial, mucilage not homogenous; individual sheaths vesicular and broad and formed one in another; cells spherical.

Gloeocapsa

ORDER NOSTOCALES

FAMILY OSCILLATORIACEAE

Trichomes, cylindrical without a sheath or single within a sheath; and sheaths open always

1. Trichomes with a prominent sheath; sheath firm; filaments not in bundles — *Lyngbya*
2. Trichomes without prominent sheath; more or less straight, not regularly spirally coiled and not in bundles — *Oscillatoria*
3. Sheath mucilagenous, filaments forming a thallus with more or less confluent sheath — *Phormidium*

FAMILY NOSTOCACEAE

1. Trichomes without firm sheath, generally not endophytic; heterocysts present — 2
1. Trichome with a firm sheath, single within a sheath; heterocysts present — 6
2. Intercalary heterocysts generally in pairs — *Anabaenopsis*
2. Intercalary heterocysts generally single — 3
3. Heterocysts commonly terminal with a single large spore adjoining — *Cylindrospermum*
3. Heterocysts terminal rarely; generally intercalary — 4
4. Filaments single or in formless gelatinous mass — *Anabaena*
4. Filaments generally in definite colonies — 5
5. Thallus finger shaped, attached at first — *Wollea*
5. Thallus otherwise — *Nostoc*

5. Cells of thallus arranged in a linear series forming pseudofilamentous growth; cells in more than a single row; thallus not cylindrical; cells with sheaths not like those of *Gloeocapsa*; with a thin sheath or without individual sheaths in a more or less homogenous gelatinous thallus; cells in distinct radial rows — *Chlorogloea*

6. Cells very short, discoid — *Nodularia*

6. Cells not discoid — *Aulosira*

FAMILY SCYTONEMATACEAE

1. Heterocysts absent; apices of trichomes as broad as the rest — *Plectonema*

1. Heterocysts present; single trichome in a sheath — 2

2. Apex not tapering — *Scytonematopsis*

2. Apex not tapering: sheath mostly with parallel lamellation — 3

3. False branches usually geminate — *Scytonema*

3. False branches usually single and often arising next to a terminal heterocyst — *Tolypothrix*

FAMILY RIVULARIACEAE

1. With terminal heterocysts; filaments in a spherical or hemispherical thallus — 2

1. With terminal heterocysts; filaments free, simple or distinctly false branched dichotomously; corymbose thallus — *Calothrix*

2. Spores not known — *Rivularia*

2. Spores commonly found, single, large — *Gloeotrichia*

ORDER STIGONEMATALES

Families: *Nostochopsidaceae*, *Mastigocladopsidaceae*, *Mastigocladaceae*, *Stigonemataceae*

1. With lateral or reverse 'V' shaped branching, pedicellate heterocysts present — 2

1. With lateral or reverse 'V' shaped branching, pedicellate heterocysts absent — 3

2.	Branching true and lateral	*Nostochopsis*
2.	Branching reverse 'V' shaped	*Mastigocladopsis*
3.	Branching reverse 'V' shaped, filaments not ending in a hair	*Mastigocladus*
3.	Branching true and lateral	4
4.	Older filaments with many rows of cells; filaments prostrate without a distinct erect system	*Stigonema*
4.	Older filaments with a single row of cells or many rows only for short portions	5
5.	Lateral branches not much different from the main filament	6
5.	Lateral branches distinct from the main filament	*Fischerella*
6.	Hormocysts present	*Westiella*
6.	Hormocysts absent	7
7.	Hormogones present	*Hapalosiphon*
7.	Hormogones not known, endospores present	*Westiellopsis*

THE CLASSIFICATION OF BGA BY RIPPKA, DERUELLES, WATERBURY, HERDMAN AND STANIER (Reproduced from J. Gen. Microbiol. 1979, 111:1-61)

The main advantage of this classification is that the differential characters proposed are both constant and readily determinable in culture material. However this classification dealt with genera only. The authors have attempted to maintain the system of generic nomenclature and the generic definitions now used by phycologists. However, when the discriminatory characters that nominally distinguish two genera are not determinable on cultures or within the range of variation of a single strain, the existing genera have been combined. Among about 150 genera described in the literature 22 only are recognized. Based on differences in structure and development five sections were recognized.

Unicellular; cells single or forming colonial aggregates held together by additional outer cell wall layers	Reproduction by binary fission or by budding		**Section I**
	Reproduction by multiple fission giving rise to small daughter cells (baeocytes), or by both multiple fission and binary fission		**Section II**
Filamentous; a trichome (chain of cells) which grows by intercalary cell division	Reproduction by random trichome breakage, by formation of hormogonia and (Sections IV and V only) sometimes by germination of akinetes	Trichome always composed only of vegetative cells	Division in only one plane **Section III**
		In the absence of combined nitrogen, trichome contains heterocysts; some also produce akinetes	Division in only one plane **Section IV**
			Division in more than one plane **Section V**

Major sub-groups of cyanobacteria

Section I: Unicellular cyanobacteria that divide by binary fission or by budding

		Division in one plane	Division in two or three planes
Reproduction by binary fission	Thylakoids absent	Sheath present *Gloeobacter*	
	Thylakoids present	Sheath present *Gloeothece*	Sheath present *Gloeocapsa*
		Sheath absent *Synechococcus*	Sheath absent *Synechocystis*
Reproduction by budding	Thylakoids present	*Chamaesiphon*	

Section II: Unicellular cyanobacteria that reproduce by multiple fission

		○ Baeocytes without fibrous outer wall layer ◎ Baeocytes with fibrous outer wall layer	
Reproduction only by multiple fission	Motile baeocytes ○ Immotile baeocytes ◎		*Dermocarpa* *Xenococcus*
Reproduction by both binary fission and multiple fission	Binary fission leads to pear-shaped structure composed of one or two basal cells and one apical cell; subsequent multiple fission of the apical cell yields motile baeocytes ○		*Dermocarpella*
	Binary fission yields cubical cellular aggregates; subsequent multiple fission yields: motile baeocytes ○ immotile baeocytes ◎		*Myxosarcina* *Chroococcidiopsis*
	Binary fission yields irregular cellular aggregates (pseudofilamentous); subsequent multiple fission yields motile baeocytes ○		Pleurocapsa group

Section III: Filamentous non-heterocystous cyanobacteria that divide in only one plane

Trichome helical	Cells composing trichome are isodiametric, cylindrical or disc-shaped; little or no constriction between adjacent cells; reproduction by transcellular trichome breakage (?)	Trichome motile, either not ensheathed or thinly sheathed *Spirulina*
Trichome straight	Cells composing trichome are disc-shaped and not separated by deep constrictions; reproduction by transcellular trichome breakage	Trichome motile, either not ensheathed or thinly sheathed *Oscillatoria*
		Trichome immotile, enclosed by heavy sheath; motility restricted to sheathless or thinly sheathed hormogonia LPP group A
	Cells composing trichome are isodiametric or cylindrical; variable degree of constriction between adjacent cells; reproduction by transcellular or intercellular trichome breakage	Trichome motile, not ensheathed; cells contain polar gas vacuoles and are separated by deep constrictions *Pseudanabaena*
		Not as above; with or without sheath, motile or immotile LPP group B

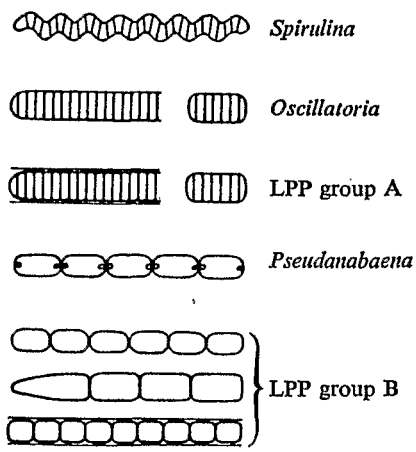


Diagram 1. Schematic presentation of the genera assigned to Section III. Thin lines surrounding trichomes designate sheath material. Polar bodies (*Pseudanabaena*) represent gas vacuoles.

Section IV: Filamentous heterocystous cyanobacteria that divide in only one plane

Reproduction by random trichome breakage, and (in some) by germination of akinetes, to produce trichomes indistinguishable from the mature vegetative trichomes	Heterocysts are intercalary or terminal; position of akinetes (if produced) is variable	Vegetative cells are spherical, ovoid or cylindrical *Anabaena*
		Vegetative cells are disc-shaped *Nodularia*
	Heterocysts are exclusively terminal and are formed at both ends of the trichome; akinetes are always adjacent to heterocysts	Vegetative cells are isodiametric or cylindrical *Cylindrospermum*
Reproduction as above, and also by formation of hormogonia, distinguishable from mature trichomes by the absence of heterocysts and by one or more of the following characters: rapid gliding motility, smaller cell size, cell shape and gas vacuolation	Hormogonia give rise to young filaments that bear a terminal heterocyst at both ends of the cellular chain	Vegetative cells are spherical, ovoid or cylindrical; akinetes (if produced) are not initiated adjacent to heterocysts and are often formed in chains *Nostoc*
	Hormogonia give rise to young filaments that bear a terminal heterocyst at only one end of the cellular chain	Mature trichome is composed of cells of even width; heterocysts are predominantly intercalary; vegetative cells are disc-shaped, isodiametric or cylindrical *Scytonema*

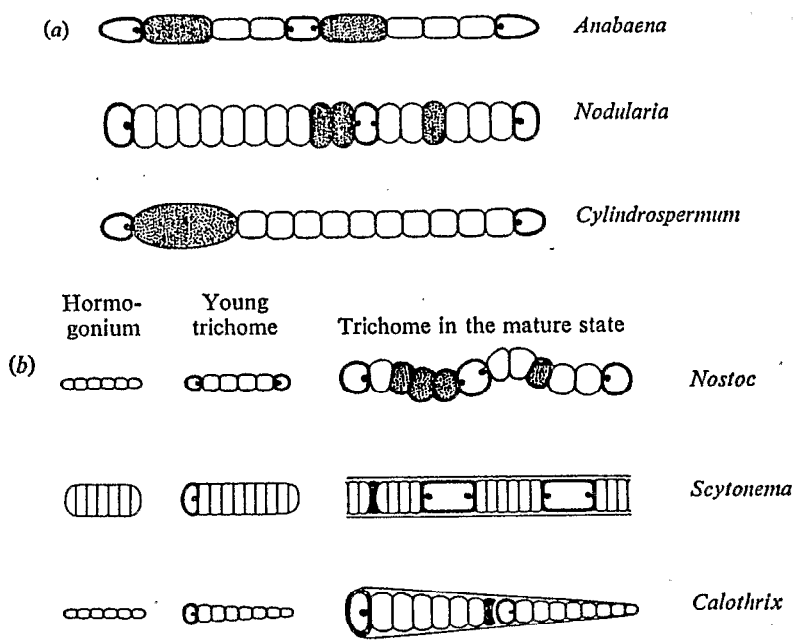


Diagram 2. Schematic presentation of the genera assigned to Section IV: (*a*) without developmental cycle; (*b*) with developmental cycle. Heavy walled cells with polar granules represent heterocysts; heavy walled cells that are dotted represent akinetes; thin lines surrounding trichomes designate sheath material.

Section V: Filamentous heterocystous cyanobacteria that divide in more than one plane

Reproduction by random trichome breakage, by formation of hormogonia and (if produced) by germination of akinetes	Hormogonia composed of small cylindrical cells which enlarge and become spherical; heterocysts develop in terminal and intercalary positions	Cells in the mature trichome divide in more than one plane; associated detachment of groups of cells leads to irregular *Gloeocapsa*-like aggregates containing terminal heterocysts; hormogonia are produced within such aggregates *Chlorogloeopsis*
	Hormogonia composed of small cylindrical cells which enlarge and become rounded; heterocysts develop almost exclusively in an intercalary position	Cells in the mature trichome divide in more than one plane to produce a partly multiseriate trichome with lateral uniseriate branches; heterocysts in the primary trichome are predominantly terminal or lateral; hormogonia are produced from the ends of trichomes or from lateral branches *Fischerella*

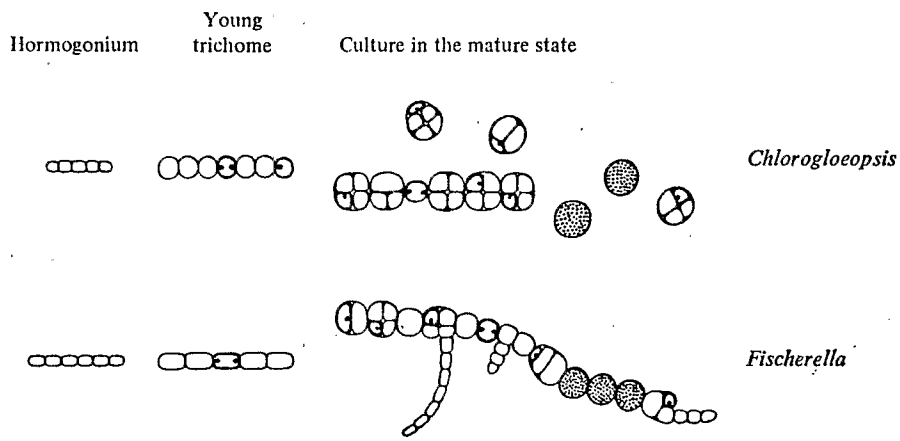


Diagram 3. Schematic presentation of the genera assigned to Section V. Heavy walled cells with polar granules represent heterocysts; heavy walled cells that are dotted represent akinetes; thin lines surrounding groups of cells designate sheath material.

2 PHYSIOLOGY OF BLUE-GREEN ALGAE

21 CARBON NUTRITION

BGA are capable of adopting different modes of carbon metabolism.

Photosynthesis, the manufacture of organic substances from CO_2 and H_2O in the light, is the main method of nutrition in BGA (autotrophy).

$$CO_2 + H_2O \xrightarrow{\text{light}} CH_2O + O_2$$

Carbon dioxide + water ⟶ carbohydrates + oxygen

However, when provided with organic compounds in addition to CO_2, many BGA utilize organic carbon in cell synthesis. There are also few species which can grown in the dark at the expense of organic carbon source (heterotrophy).

22 NITROGEN NUTRITION

Blue-green algae can readily utilize inorganic nitrogen compounds such as nitrate, nitrite and ammonium salts. Some species can also assimilate elemental nitrogen (N_2) from the atmosphere (N_2-fixing species), and certain species can use organic nitrogen compounds.

Ammonium-nitrogen, although energetically the most favorable nitrogen-source, often supports poorer growth than does nitrate supplied at comparable level and may cause cell lysis. Nitrate nitrogen is the most preferred nitrogen source in most culture media.

221 Nitrogen Fixation

Nitrogen fixation is the incorporation of atmospheric nitrogen as a nitrogen source into the cells of an organism. The ability to fix nitrogen is restricted to some of the prokaryotic microorganism. Among BGA only 27 genus are N_2-fixing. Following table reproduced from WDP Stewart (Systems involving BGA (cyanobacteria) in "Methods for Evaluating Biological Nitrogen Fixation", BERGERSEN ed) gives the current position regarding N_2-fixing BGA.

Table 2

Nitrogen-fixing Cyanobacteria

Group	Genus	Total tested	Aerobic nitrogenase	Anaerobic/ microaerobic nitrogenase	Assay condition*
Chroococcacean	*Aphanothece*	1	1	1	T.N.
	Gloeothece†	5	5	5	C_2H_2
	Synechococcus	27	0	3	C_2H_2
Pleurocapsalean	*Dermocarpa*	6	0	2	C_2H_2
	Xenococcus	3	0	1	C_2H_2
	Myxosarcina	2	0	1	C_2H_2
	Chroococcidiopsis	8	0	8	C_2H_2
	Pleurocapsa	12	0	7	C_2H_2
Non-heterocystous‡ filamentous forms	*Oscillatoria*	9	0	5	C_2H_2
	Pseudanabaena	8	0	4	C_2H_2
	Lyngbya–Plectonema–Phormidium	25	0	16	C_2H_2, $^{15}N_2$, T.N.
Heterocystous filamentous forms	*Anabaena*	15	15	15	C_2H_2, $^{15}N_2$, T.N.
	Anabaenopsis	2	2	2	C_2H_2, $^{15}N_2$, T.N.
	Aulosira	1	1	1	T.N.
	Calothrix	4	4	4	C_2H_2, $^{15}N_2$, T.N.
	Cylindrospermum	5	5	5	C_2H_2, T.N.
	Fischerella	2	2	2	T.N.
	Hapalosiphon	1	1	1	T.N.
	Mastigocladus	1	1	1	T.N.
	Nostoc	13	13	13	C_2H_2, $^{15}N_2$, T.N.
	Scytonema	3	3	3	T.N.
	Stigonema	1	1	1	T.N.
	Tolypothrix	2	2	2	T.N.
	Westiella	1	1	1	T.N.
	Westiellopsis	1	1	1	$^{15}N_2$, T.N.

*Certain, or all of the cyanobacteria have been tested by these methods; T.N. = total nitrogen.
†Includes strains previously designated as N_2-fixing *Gloeocapsa* strains.
‡The data given here are those of Rippka and Waterbury (1977), but the exact numbers of strains tested and shown to have nitrogenase may be larger since various earlier workers (Stewart and Lex, 1970; Stewart, 1970; Stewart *et al.*, 1977) had examined and obtained positive results with strains which may or may not correspond to those tested by Rippka and Waterbury (1977). (From Stewart *et al.*, 1979.)

All heterocystous forms fix nitrogen, both in aerobiosis and anaerobiosis. Some non-heterocystous (homocystous) filamentous forms fix nitrogen in anaerobiosis. A few unicellular forms fix nitrogen in anaerobiosis; among these Aphanothece and Gloeothece are also able to fix nitrogen in aerobiosis.

Nitrogen-fixing activity occurs only under mineral nitrogen starvation. In heterocystous BGA, heterocyst formation is inhibited by combined nitrogen sources like nitrate and ammonium.

222 Nitrogenase

Nitrogenase is the enzyme involved in nitrogen fixation.

$$N_2 + 3H_2 \xrightarrow{\text{nitrogenase}} 2NH_3$$

Nitrogenase is not specific of nitrogen and can reduce a variety of triple bond ($N \equiv N$) substitutes like acetylene.

$$C_2H_2 + H_2 \xrightarrow{\text{nitrogenase}} C_2H_4$$

acetylene + hydrogen ethylene

This property is used for evaluating nitrogenase activity with the acetylene reduction method.

Nitrogenase is oxygen labile.

223 Relations between photosynthesis and nitrogen fixation

As pointed above, nitrogenase is irreversibly inactivated by oxygen. Therefore, aerobic nitrogen fixation and O_2 producing photosynthesis are two process which cannot occur simultaneously

in the same cell.

In heterocystous BGA, the heterocyst is the sole site of nitrogen fixation under aerobic conditions. It provides a protective site for nitrogenase because it lacks photosystem II (the photosystem responsible for CO_2 fixation and O_2 production) but has nitrogenase and photosystem I which provides energy for the heterocyst. Heterocysts fix nitrogen to ammonia and to glutamine which is then supplied to vegetative cells, while vegetative cells in return supply fixed carbon to the heterocysts (Fig. 1).

23 OTHER NUTRIENTS

Besides C, H, N, and O, the major elements required for the growth of BGA (P, S. K, Na, Mg, Ca) are not different from the requirements of other plant groups. Trace elements (Fe, Mn, Bo, Mo, Cu, Zn, Co) are also essential for the growth of BGA. Molybdenum which is a component of both nitrogenase and nitrate reductase is essential for nitrogenous nutrition of BGA.

3 ECOLOGY OF BLUE-GREEN ALGAE IN PADDY FIELDS

31 METHODS

311 Evaluation of algal abundance

Three techniques for evaluating algal abundance in soils are generally cited: plating of soil suspension, direct count, and pigment analysis.

Pigment analysis gives no indication of species and because of contamination by humic acids and chlorophyll degradation products, can be used only to compare the effect of different treatments on the total flora in the same soil.

Direct examination and counting is time consuming and boring. Moreover, because a homogenized soil suspension is used, algal filaments are more or less broken in pieces and the determination of species may be impossible in some cases.

Dilution and plating technique permits simultaneously enumeration, identification and isolation of the different algae of the sample. The main disadvantage of the plating method is that it does not ensure that all species present in the soil develops on the plates and even if they grow, their relative frequency may change. Despite its disadvantages the plating method is the most widely used.

312 Evaluation of nitrogen-fixing activity

Nitrogen analysis by the Kjeldahl technique is used in nitrogen balance studies. The use of this method to distinguish between phototrophic nitrogen fixation by parallel light and

dark treatment is suitable only for long-term trials for gross measurement.

^{15}N techniques are expensive and difficult to use in the field.

Acetylene reduction is now widely used for measuring photosynthetic NFA. It is the most useful technique for routine studies on the nitrogenase activity. Measurements of algal acetylene reducing activity are reliable when the incubation is brief, the problem of gas diffusion and greenhouse effects are minimized, and statistically valid sampling method are adopted. However the method is generally considered suitable for qualitative estimate only.

313 Sampling problems in relation to the distributional ecology of BGA

The validity and accuracy of algal enumerations and ARA measurement in the field depend on the heterogenecity of the distribution of the organisms and the density of sampling.

BGA populations have an uneven distribution that approximates a log-normal pattern, therefore a high density of sampling is needed.

A large number of small core samples is preferable to a few large samples for both algal enumerations and acetylene reducing activity measurement.

Method of sampling used at IRRI consist in taking 13 cm core samples, 2 cm in diameter, in 4 x 4 m plots. Each treatment is replicated four times.

32 OCCURRENCE OF BGA IN PADDY FIELDS

N_2-fixing BGA are not invariably isolated from tropical rice fields. In India, VENKATARAMAN reported that out of 2313 soil samples only about 33% were found to harbour N_2-fixing forms.

Fragmentary quantitative measurements indicate that N_2-fixing BGA population densities vary from a few to 10^7/g dry soil (Table 3); biomass vary from a few kilograms to 24 tons (fresh weight)/ha (Table 4). Because of variability of the water content in BGA (Table 5) fresh weight has however little significance. From the highest biomass recorded in terms of dry weight per hectare, it appears that under favorable conditions an N_2-fixing algal bloom may contribute 30-40 kg N/ha to the ecosystem.

33 FACTORS AFFECTING BGA GROWTH IN PADDY FIEDLS

331 Climatic factors

Among the climatic factors affecting the seasonal fluctuations of the phytoplankton, light is most important. It affects the algal biomass qualitatively and quantitatively. BGA may be regarded as low light species; in area of high incident light intensities BGA develop only when protected by a sufficiently dense rice canopy (Fig. 2a). In areas of moderate incident light intensities, during rainy or cloudy weather light deficiency under a dense rice canopy may limit BGA growth (Fig. 2b). To a lesser extent temperature and water regime may also influence BGA growth in the wetland field.

Table 3

References reporting algal enumerations in rice fields.

References	Location	Values (no.·g^{-1} dry soil)	Remarks
21	Thailand	10 to 10^4 [a]	9 soil types studied
22	Thailand	$\cong 10^3$ [a]	103 sites studied
71	Senegal	0 to 10^6 [a]	40 soils studied during the dry season
107	Japan	10^6 [a]	Fertilized plots
122	Thailand	10^3 [a] to 10^5 [a]	
	Malaysia	10^4 to 10^7 [a]	
	Philippines	10^3 to 10^5 [a]	
156 to 160	Thailand	$\cong 10^2$ [a]	Brackish water, alluvial soil and Regosol
		$\cong 10^3$ [a]	Noncalcic brown soil
		$\cong 10^4$ [a]	9 other soil types
304	Mali	10^3 to 10^6 [b]	12 measurements in the same field along a 2-year period.
262	India	$2 \times 10^7 \cdot cm^{-2}$ [c]	*Aphanothece pallida* from the water surface

[a]Most-probable-number method. [b]Plating method. [c]Method not indicated.

Table 4

References reporting algal biomass measurements in rice fields.

Reference	Location	Dry wt (kg·ha^{-1})	Fresh wt (kg·ha^{-1})	Remarks
2	China		7,500	After inoculation
147	India	3 to 300	60 to 6,000[a]	Green algae dominant
		32	600	N_2-fixing BGA dominant
176	UzbSSR		16,000	Total algal biomass
219	Senegal		2 to 6,000	Total algal biomass
			2 to 2,300	N_2-fixing algal biomass
239	Philippines	2 to 114		
261	India	480	9,000[a]	*Aulosira* bloom
280	India		100 to 2,100	
353	Philippines	177	24,000	*Gloeotrichia* bloom

[a]Data extrapolated on the basis of 95% water content.

Table 5

Mean composition of different BGA genera and biomasses corresponding to 10 kg N·ha^{-1}

Genera	Strains tested (no.)	Dry matter (% of fresh wt)	Protein (% of dry wt)	Fresh wt (t·ha^{-1}) corresponding to 10 kg N·ha^{-1}
Calothrix	6	5	32	3.9
Nostoc	4	2.2	30	9.4
Gloeotrichia	8	0.74	29	29.0

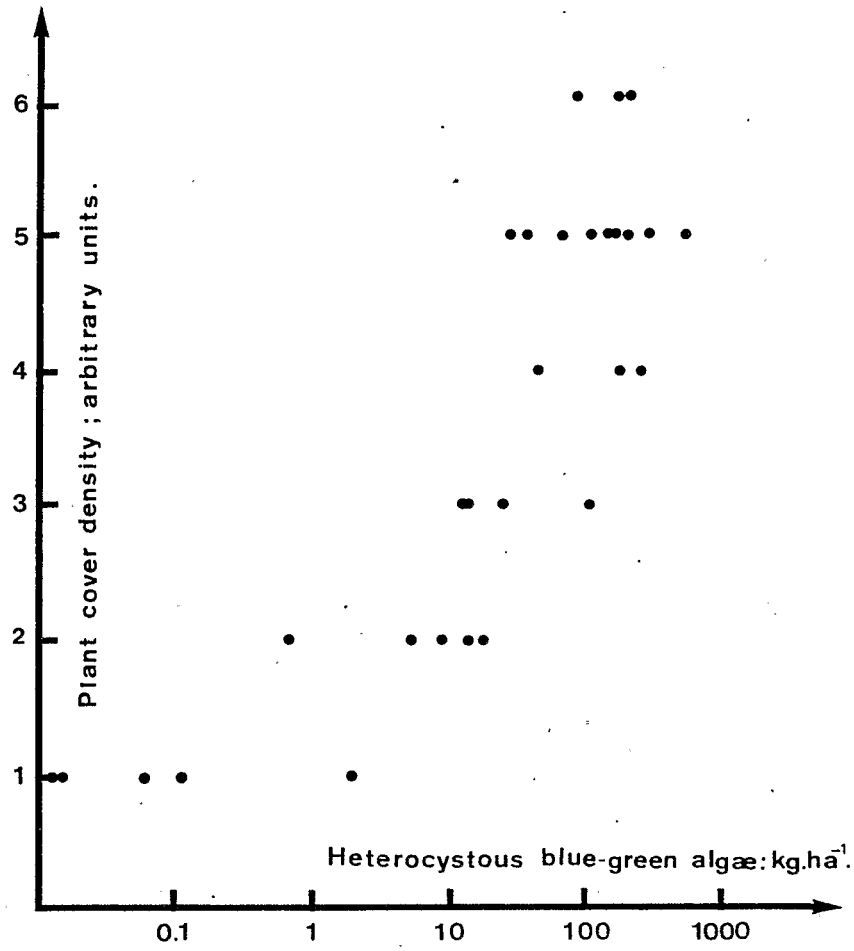
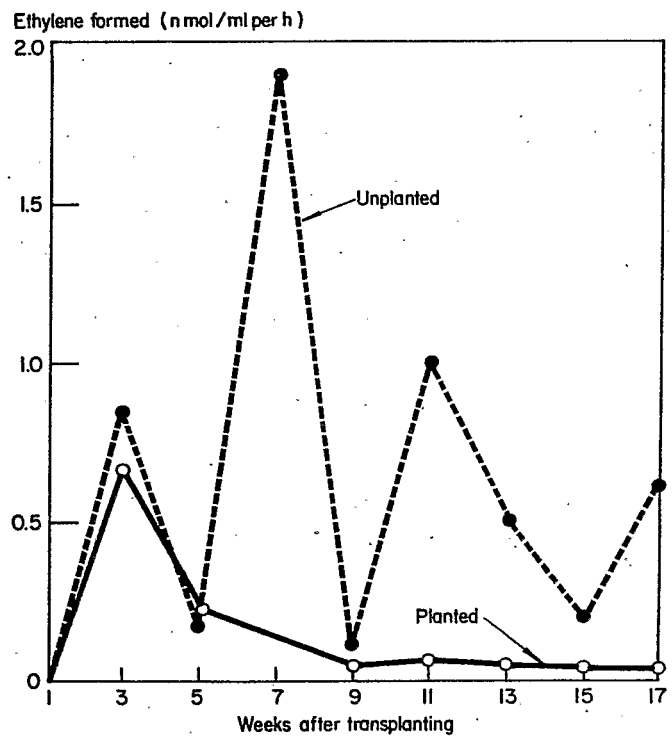


Fig. 2a

In Senegal (West Africa) where light intensity is very high (> 80,000 lux at 12.00) along the whole year, N_2-fixing BGA develops only when the rice canopy is sufficiently dense to protect them from the detrimental light. There is a positive correlation between the density of the plant cover and the N_2-fixing algal biomass.



1. Variations of algal ARA in paddy water during cultivation cycles at IRRI; cropped and noncropped fields during the wet season (Yoshida and Ancajas 1973).

Fig. 2b.

In the Philippines, during the wet season, incident light intensities are low and after tillering light intensity under the rice canopy is very low. This explains why NFA is lower in planted soil than in unplanted soil during the wet season.

332 Physico-chemical factors

Among soil properties, pH is certainly the most important factor determining the algal flora composition. Under natural conditions, BGA grow preferentially in environments that are neutral to alkaline. A common observation is a positive correlation between pH and occurrence of BGA. Next to pH, the most decisive factor favoring BGA growth is the available phosphorus content of the soil.

Other nutrients (mo, Fe, Mg, K, etc.) are required for optimal growth of BGA, but their ecological implications as limiting factors or as factors affecting the composition of the algal community in paddy fields have not been documented. Very little information is available on the effect of other soil properties on BGA.

333 Biotic factors

Among the biotic factors capable of limiting BGA growth in rice fields, only the grazing by invertebrate populations (microcrustaceans and snails) has been documented. Evidence exists that pathogenecity and antagonisms may be operative in the fields but these have not yet been demonstrated.

334 Agricultural factors

The various agronomical practices adopted along the cultivation cycle influence growth of BGA.

- Land preparation and management seem to have only incidental effects.
- Pesticides -- depending upon their nature, their concentration and the algal strains -- could have inhibitory, selective or stimulatory effects on BGA. Experiments done mainly with flask cultures suggest

that BGA were generally more resistant to pesticides than were other algae and tolerated pesticide levels recommended for field application. Insecticides are generally less toxic to BGA than other pesticides and have the secondary beneficial effect of suppressing the grazer population.

- Among chemical fertilization practices, phosphorus application and liming of acidic soils have demonstrated a beneficial effect on BGA growth. The effect of nitrogenous fertilizers is not well known, and the observed inhibition on algal ARA by mineral nitrogen in flask cultures may not be effective to the same extent under natural conditions. The effect of N fertilizers in the field have received little attention. This is surprising in view of the observation that BGA inoculation produces an increase in grain yield even at high levels of fertilizer N. From experiments conducted without algal inoculation, a depressive effect of N fertilizer on algal NFA has been established.

- Depending on their nature and mode of application, organic manure may favor or depress BGA growth. Plant residue incorporation, which produce anaerobic decomposition by-products toxic to algae seems to be less beneficial to BGA than surface application.

34 ALGAL SUCCESSIONS IN PADDY FIELDS

Algal populations appear to be highly susceptible to environmental changes and exhibit rapid qualitative and quantitative variations along the cultivation cycle.

Table 6 and Figure 3 are an example of the evolution of the algal flora in paddy fields in Senegal. During the early part of the cultivation cycle (planting and tillering), the algal biomass increased and

Table 6

Algal biomass composition in relation to rice development in 40 paddy fields in Senegal (Reynaud and Roger, 219).

Stages of rice development	Dominant flora				N_2-fixing algae (% of total biomass)		
	Nature	% of total biomass					
		Mean value	Max value	Min value	Mean value	Max value	Min value
Tillering	Diatoms, unicellular green algae	73	99	49	2	4	0.1
Panicle initiation	Filamentous green algae, non-heterocystous blue-green algae	89	93	86	3	9	0.1
Heading to maturity Weak plant cover	Filamentous green algae, non-heterocystous blue-green algae	70	91	62	8	14	0.2
Heading to maturity Dense plant cover	Blue-green algae	71	99	16	38	99	13.0

A

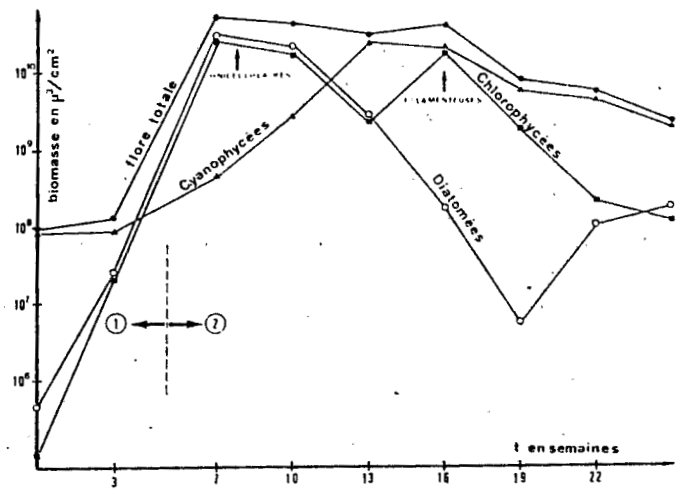


FIG. 3. — Variations des biomasses des différents composants de la flore algale totale au cours du cycle de végétation : flore totale : ●——● ; Diatomées : ○——○ ; Chlorophycées : ■——■ ; Cyanophycées : ▲——▲ (1) et (2) voir explication dans le texte.

B

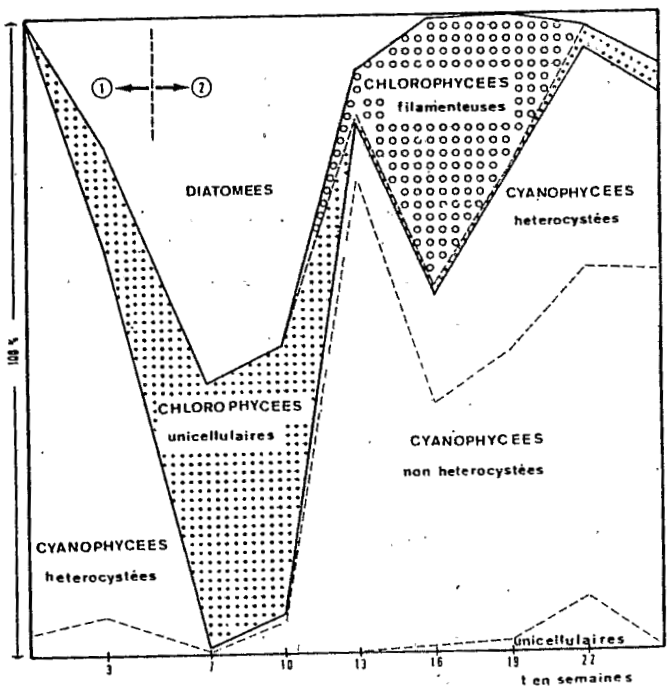


Fig. 3 a and b

Variations of the components of the components of the algal flora in a paddy field in Senegal.

a: absolute values

b: relative values

Cyanophycees = BGA

Chlorophycees = Green algae

Data plotted on the left side of the dotted line (1) correspond to the two first enumerations done on the dry soil and just after rewetting.

consisted mainly of diatoms and unicellular green algae. From tillering to panicle initiation, the algal biomass reached its highest values and filamentous green-algae and more N_2-fixing BGA were dominant. After panicle initiation the total biomass decreased. If the plant cover was sufficiently dense, heterocystous BGA developed; if it was thin, filamentous green algae and homocystous BGA remained dominant.

The following interpretation of algal flora variations was proposed: At the beginning of the cultivation cycle, paddy soils were characterized by:

- A low pH, which favored the development of chlorophyceae but not of BGA.
- An absence of plant cover and a corresponding high light intensity at the air-water interface that was also favorable for the development of chlorophyceae and diatoms but unfavorable to BGA.
- A high level of CO_2 caused by soil remoistening which favored green- algae.

During the cultivation cycle, a decrease in light intensity and N level related to rice growth and an increase in pH value favored BGA growth. The non evolution of algal flora under a weak plant cover indicated the important role of light in regulating the algal composition.

35 NITROGEN FIXATION BY BGA IN PADDY FIELDS

Algal NFA has most frequently been studied by ARA measurement. This method is certainly liable to misinterpretation of quantitative results, but it is very convenient and reliable for qualitative studies when the measurements are brief, the problems of gas diffusion and greenhouse effects are minimized, and statistically valid sampling methods are adopted.

Diurnal variations in ARA are related mainly to the variations of the light intensity. Depending on the maximal value of the light intensity during the day, the curve will exhibit one or two maxima; the second pattern correspond to an inhibitory effect of high light during the middle of the day.

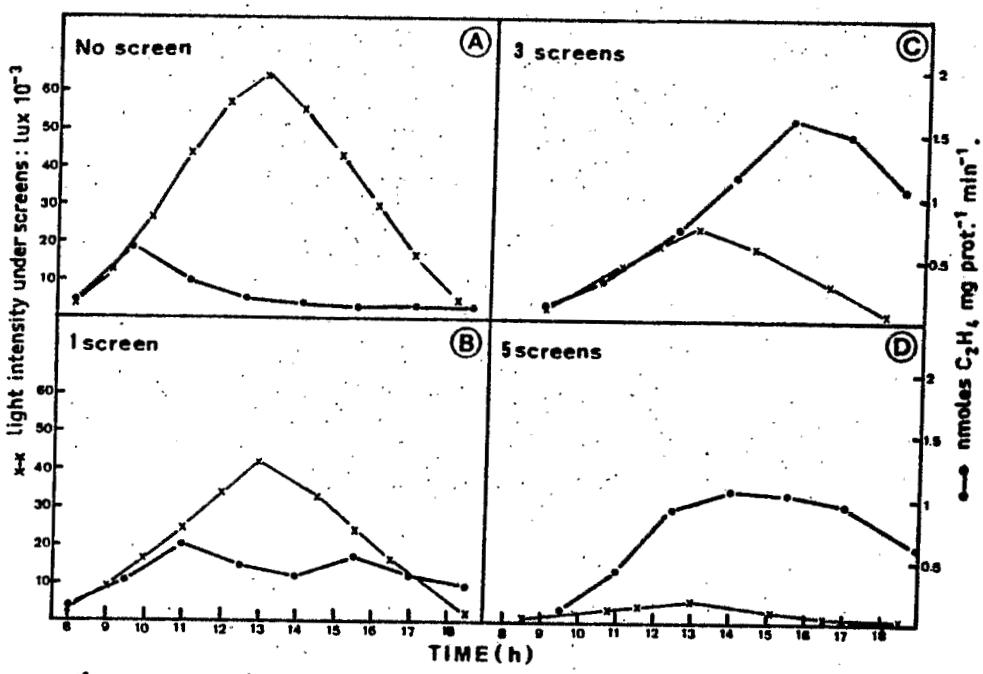


Figure 4. Diurnal variations of acetylene reduction by an *Anabaena* bloom placed under artificial plant cover.

Studies reporting variations of algal ARA along the cultivation cycle indicate that a peak of activity may occur anytime. A predominant effect of light intensity in relation to the season and the plant cover seem to be well established.

The estimated amounts of fixed nitrogen vary from a few to several kilograms per crop. The average value of the reported estimates (30 kg per crop) seems to constitute a satisfactory reference value when environmental factors favor BGA growth. The relative contribution of BGA as a percentage

of the total nitrogen fixed in the paddy field varies within large limits and seems to be more affected by nitrogen fertilizers than the heterotrophic N_2-fixation (Table 7).

Relative contribution of floodwater, plant, and soil to N_2-fixation in a paddy field (from Watanabe et al, 359).

Treatment	ARA[a] (mmol $C_2H_4 \cdot m^{-2} \cdot day^{-1}$)			
	Floodwater	Plant	Soil	Total
–NPK	0.72 (61)	0.28 (21)	0.35 (18)	1.35 (100)
+NPK	0.13 (16)	0.37 (42)	0.37 (42)	0.74 (100)

[a]Figures in parentheses indicate percent of total.

36 BLUE-GREEN ALGAE AND THE RICE PLANT

Detrimental effects:

Among the algae detrimental to rice, because of their mechanical effect on the young plants, BGA can be considered as incidental. Even where they produced a bloom at the beginning of the cultivation cycle, their effect on yield was rarely negative.

Exudation products:

BGA exudate O_2, organic acids, growth promoting substances and nitrogen. Oxygen production by BGA in very anaerobic soils may prevent development of sulphate reduction processes harmful to rice. Organic acids increase phosphorus availability.

Besides increasing nitrogen fertility, BGA have benefited rice plants by the production of growth-promoting substances. The additive effect of algalization in the presence of a high level of fertilizer N was interpreted as an index of this growth promoting effect, but such an interpretation has

still not been demonstrated in the field and has to be treated with caution. Part of fixed nitrogen is exuded into the floodwater. Availability of exudated nitrogen to the rice plant is certainly poor except in the case of deepwater rice where BGA growth epiphytically on the rice plant especially on the submerged roots. Experiments conducted at IRRI demonstrated that BGA epiphytism makes a limited contribution to the nitrogen input in shallow water rice, but this contribution has agronomic significance in deepwater rice.

Retarded action:

Nutrients fixed by BGA are released through microbial decomposition after the cells die. Experiments conducted at IRRI with ^{15}N labeled BGA showed that availability of N from dried BGA incorporated in the soil was between 23-28% for the first crop and 27-36% for two crops. Surface application of the algal material reduced the availability to 14-23% for the first crop and 21-27% for two crops. Availability of algal N to the current and following crop was almost equal to availability of ammonium sulfate to the first crop. Due to its organic nature BGA material is less susceptible to N losses than inorganic fertilizers. However, its low C/N ratio (5-6) gives it a better N availability than those of organic fertilizers like farm yard manure.

37 CULTURAL PRACTICES TO INCREASE BGA GROWTH AND NFA IN THE FIELD

- Liming of acidic soils
- Phosphorus application
- Molybdenum application
- Deep placement of N fertilizers
- Control of grazers population
- Surface application of rice straw
- Algal inoculation (algalization)

4 ALGALIZATION

Since BGA were recognized to be one, if not the most, important N_2 fixing agent in flooded rice soils, many trials have been conducted to increase rice yield by algal inoculation (algalization). Unfortunately most of the experiments have been conducted on a "black box" basis where only the last indirect effect (grain yield) of an agronomic practice (algalization) was observed and the intermediate effects were not studied. Very little information is available on the qualitative and quantitative evolution of the N_2-fixing algal flora, the evolution of the photosynthetic NFA, and the nitrogen balance in inoculated paddy soils. Pot experiment may be suitable for qualitative studies, but overestimate the effects of algal inoculation. On the other hand, most of the field experiments have been conducted over one growing season only and may underestimate the effects of algalization. The advantages of a slow N release might not be apparent in the first crop after algal inoculation.

Table 8

Reproduced from Roger and Kulasooriya - 1980.

Relative increase in grain yield over the treatment in pot and field experiments.

	Pot experiments	Field experiments
	Experiments comparing both methods[a]	
Mean	28.1	15.2
Standard deviation	33.2	12.3
Number of data	22	23
	All experiments listed in Table 17	
Mean	42.0	14.5
Standard deviation	59.6	8.9
Number of data	64	102

[a]Reference no. 94, 112, 143, 212, 213, 286 287.

Algalization has been reported to have a beneficial effect on grain yield in several countries however there are also reports indicating a failure of algalization under widely different agroclimatic conditions. Little is known about the limiting factors for algalization. Among the soil properties, a low pH and a low available phosphorus content are the only well documented ones. Knowledge of the relation between soil properties and the establishment of the algal inoculum is certainly a major gap. Among detrimental biotic factors only grazing by zooplankton has been studied. Low temperature, heavy rains and cloudy weather have also been reported to limit the establishment of the algal inoculum.

Table 9

Average value of grain yield in algalization experiments reported in the literature. (Tables included in this paragraph are reproduced from Roger and Kulasooriya. "Blue-green algae and rice" 1980).

Experimental	Grain yield in the control ($kg \cdot ha^{-1}$)	Number of		Variation in grain yield due to					Geographical location
				Algalization			Treatment		
				Relative (%)		Absolute ($kg \cdot ha^{-1}$)	Relative (%)	Absolute ($kg \cdot ha^{-1}$)	
		Crops	Sites	Over the treatment	Over the control	Over the treatment	Over the control		
Average in the pot experiments									India: 30
Mean	–			42.0	30.75	–	66.67	–	Japan: 5
Standard deviation	–			59.6	40.3	–	72.48	–	China: 3
Number of data	–			64	49	–	37	–	Egypt: 3
									Phil.: 1
									USSR: 1
Average in the field experiments:									
Mean	3016			14.5	16.1	475	30.0	917	
Standard deviation	803			8.9	9.3	274	22.2	698	
Number of data	30			102	87	80	51	47	
Average in the field experiments in absence of nitrogen fertilizer									
Mean	2979			14.6	16.4	442	27.9	716	
Standard deviation	789			10.4	11.6	267	20.0	436	
Number of data	25			39	31	36	17	13	
Average in the field experiments in presence of nitrogen fertilizer									
Mean	3434			14.3	14.9	488	32.2	1038	
Standard deviation	867			11.8	8.4	269	23.8	766	
Number of data	13			44	36	38	32	32	

Algalization, when effective, has been reported to increase the size of the plant, its nitrogen content, and the number of tillers, panicles, spikelets, and filled spikelets per panicle. The better grain yield has been used to assess the effect of algal inoculation. From the reports on fields experiments, conducted mainly in India, it appears that on average, algal inoculation, where effective, causes about 14% relative increase in yield, corresponding to about 450 kg grain/hectare per crop.

Table 10

Effect of lime, phosphorus, and molybdenum application, in combination with algalization, on grain yield in field experiments.

Reference no.	Treatment	Yield in the control (kg grain·ha^{-1})	Increase in yield (kg grain·ha^{-1})		
			Over the control when inoculated	Over the control due to the treatment	Over the treatment when inoculated
112	P (?)	2008	743	38	976
126	L (?)	3372	49	343	170
212	P (67)	2916	561	863	–62
	P (67) Mo (0.28)	2916	561	837	123
	L (2242)	2916	561	413	470
	L (2242) Mo (0.28)	2916	561	393	541
	P (67) L (2242) Mo (0.28)	2916	561	1833	378
	Mo (0.28)	2916	561	444	189
213	P (20) L (1000) Mo (0.28)	2379	199	563	499
	R + P (20) L (1000) Mo (0.28)	2379	199	1110	172
216	P (20) L (500) Mo (0.28)	2587	–	–	357
242	P (20) L (1000) Mo (0.28)	1536	274	708	555
	R + P (20) L (1000) Mo (0.28)	1537	274	1028	291
Number of data		13	12	12	13
Mean		2561	425	714	358
Standard deviation		569	213	470	262

A higher increase in grain yield was observed when algalization was done in combination with lime, phosphorus, and sometimes molybdenum application. It appears however, that the increase in yield strictly due to algalization does not significantly differ in the presence or absence of non-nitrogen fertilizers and that the increase in yield due to non nitrogenous fertilizers is generally higher than that due to algalization.

Results concerning the effects of algalization in the presence of nitrogen fertilizers are controversial. Several reports indicate a failure of algalization in the presence of fertilizer N. On the other hand, a large scale experiment conducted in India indicates a beneficial effect of algalization even at very high levels of nitrogen.

Table 11

Absolute variation in grain yield over the treatments due to algalization at different levels of fertilizer N in field experiments.

Reference no.	Yield (kg grain•ha^{-1})				
	No nitrogen	25–30 kg N•ha^{-1}	50–60 kg N•ha^{-1}	75–100 kg N•ha^{-1}	120–150 kg N•ha^{-1}
7	?	815	453	597	715
7	?	636	743	353	696
13	489	690	333	216	155
109	103	474	227	–	–
111	798	–	846	730	678
111	656	–	1004	770	530
243	472	49	–	–	–
255	831	–	856	–	498
271	676	641	814	–	–
271	332	93	225	–	–
273	442	626	652	–	–
275	–	–	–	393	–
324	610	–	–	–	440
331	–	–	–	373	–

There is evidence that algalization produces both a cumulative and residual effect attributed to a buildup of the soil nitrogen, organic matter, and the algal flora. However, little is known about the effects of algalization on soil properties and soil microflora.

Table 12

References reporting cumulative effect of algalization on grain yield.

Reference no.	Location	Relative increase in grain yield (% over the control)					Remarks
		1st yr	2nd yr	3rd yr	4th yr	5th yr	
344	Japan	2	8	15	19.5	10.6	Average of 9 field experiments on nitrogen-poor soils
337	Japan	2.7	8.4	19.1	21.8		Field experiment
90	Japan	5	10	15	20	–	Field experiment
214	India	3.6	20	–	–	–	Pot experiment
		44.3	190	–	–	–	Pot experiment, P and Mo added
		8	43				Field experiment
		21	41	–	–	–	Field experiment, lime, P, and Mo added

Another gap concerns the comparison between algalization and management practices enhancing growth and activity of indigenous natural population of N_2-fixing BGA. In some cases, the latter can make algalization unnecessary. Algalization is necessary where efficient strains are absent in the soil. The search for highly efficient strains is still at a theoretical level, therefore, the recommended inoculum is a soil based mixture of strains.

Algalization in rice fields has proceeded a little beyond the stage of fundamental research, and attempts have been made to popularize this technology among Indian farmers. A method for producing algal inoculum, easily adoptable by farmers, has been developed and recommendation for field inoculation given. The economics of inoculum production and pay off from the algalization technology have indicated a cost-benefit ratio working out to 1 to 10 and an additional income of about 300 Indian rupees per hectare and per crop (1979). To our knowledge, such trials are still confined to India.

41 TECHNOLOGY OF ALGALIZATION

The following is reproduced from G. S. Venkataramam "Blue-green algae for rice production". FAO Soils Bulletin No. 46. 1981. The original text comprises pictures that have not been reproduced here.

PRODUCTION OF ALGAE FOR FIELD APPLICATION

The introduction of algal biofertilizer as a package of practices in rice cultivation will depend largely upon an efficient and economical system of large-scale production of blue-green algae, its preservation and transport. A number of methods have been suggested for this (Venkataraman 1972).

A simple, rural-oriented, open-air method for producing these algae has been developed at the Indian Agricultural Research Institute at New Delhi and this method is being used by many farmers and agencies in India. The method is credited with simplicity of operation and adaptability by small and marginal farmers. The algae used in this system is a mixture of species of *Tolypothrix, Aulosira, Anabaena, Nostoc and Plectonema*. The same system is reported to be working well in Burma.

The procedure, starting in the laboratory and ending in the field, is described below. It may be noted that other efficient strains of algae may be selected and used, depending on the regions and locations.

Laboratory Culture

i. Maintain stock cultures of different nitrogen-fixing blue-green algae on 1 to 1.5% agar slants.

ii. Maintain the same cultures also in a soil extract medium (1 g of soil + 10 cm^3 of Fogg's medium, sterilized together in test-tubes). For Fogg's medium and soil extract medium see Appendix V.

iii. Grow the algae in 250 cm^3 flasks containing 100 cm^3 of Fogg's medium in the light.

iv. Scale up the cultures in aspirator bottles or carboys.

v. Transfer the algae to troughs to prepare soil-based starter material as described later under 'Trough Method'.

Different algal forms are cultured and maintained separately before transferring them to the troughs. The pH of all the cultures should be between 7.0 and 7.5 unless acid or alkali-tolerant forms are used. All the cultures are grown in light.

Trough Method

i. Prepare shallow trays (2 m x 23 cm) of galvanized iron sheet (Figure 6) or permanent tanks . The size can be increased if more material is to be produced.

ii. Introduce 8 - 10 kg of soil and mix well with 200 g of superphosphate

iii. Place from 5 to 15 cm of water in the trays depending on the local conditions and rate of evaporation. The reaction of the soil should be about neutral; if acidic correct by adding lime

iv. To prevent insects, add Carbofuron (3% granules) at the rate of 25 g per tray or BHC or other suitable insecticide.

v. After the soil has settled, sprinkle the algal culture on the surface of the standing water (Figure 14). Keep the units in the open air and completely exposed to the sun.

vi. In hot summer months, the growth of the algae will be rapid and in about seven to ten days they form a thick mat (Figures 7 and 8). If the daily rate of evaporation is high, add water intermittently. When the algal growth becomes sufficiently thick, stop watering.

vii. Allow the water to evaporate completely in the sun the dry algae cracks into flakes.

viii. Collect the dry algal flakes from the trays and store in bags for use in the fields

ix. Fill the troughs again with water and add a small amount of the dry algal flakes as further inoculum. Continue the process as above. Once the soil in the troughs is exhausted (usually after three or four harvests) replace it with fresh soil mixed with superphosphate and continue as above. A single harvest of surface algae from one trough of the given dimensions will give about 1.5 to 2.0 kg of material.

Pit Method

This method does not differ from the trough method except in magnitude. Instead of troughs or tanks, shallow pits are dug in the ground and layered with a thick polythene sheet to hold the water . Other procedures are the same as in the trough method. This method is easy and less expensive to operate by small farmers.

The experiments conducted at the Paddy Experiment Station at Aduthurai in Tamil Nadu and at the Soil Testing Laboratory at Anakapalli in Andhra Pradesh in India show that addition of sawdust or rice husk to the troughs or pits at the rate of 200 g enhances the biomass production. This is presumed to be due to the availability of a greater surface area and since algae cling to these materials harvesting also becomes easier. Also the accidental collection of excess soil along with the algae during harvesting is avoided. Sometimes the addition of 200 g of superphosphate as a single dose results in problems of acidity. To avoid this, split the dose into two or three smaller doses; similarly the amount of soil can be split.

Field Production

The field production of algae is really a scaled up operation of the trough or pit methods to produce the material on a commercial scale: it is being adopted by a number of farmers in south India.

i. Demarcate the area in the field for algal production; the suggested area is 40 m^2. No special preparation is necessary although if algal production is envisaged immediately after crop harvest, the stubble is to be removed and if the soil is loamy it should be well puddled to facilitate waterlogging.

ii. Bund the area with strong 15 cm earth bunds

iii. Flood the area with water to a depth of about 2.5 cm In the trough and pit methods, flooding is done only in the beginning; in the field, flooding is repeatedly needed to keep the water standing.

iv. Apply superphosphate at 12 kg 40 m^{-2}

v. If the field has previously received algal application for at least two consecutive cropping seasons, no fresh algal application is required. Otherwise, apply the composite algal culture at 5 kg 40 m^{-2}

vi. To control predators like daphnids, snails and mosquitoes, apply Carbofuron (3% granules) or Ekalux (5% granules) at 250 g 40 m^{-2} or BHC or Furadon

vii. In clayey soils, good growth of algae takes place in about two weeks in clear, sunny weather, while in loamy soils its takes about three to four weeks

viii. Once the algae have grown and form floating mats, they are allowed to dry in the sun in the field and the dried algal flakes are then collected and stored in sacks for further use

ix. One can continually harvest algae from the same area by reflooding the plot and applying superphosphate and pesticides.

x. Addition of algal inoculum for subsequent production is not necessary.

xi. During summer months (April-June), the average yield of algae per harvest ranges from 16 - 30 kg per 40 m^2. Adopting this method, a record production of 15.6 tonnes/ha of wet blue-green algae has been obtained by farmers within three weeks.

Nursery-cum-algal Production

Farmers can produce algae along with seedlings in their nurseries. If 320 m^2 of land are allotted to prepare a nursery, an additional 40 m^2 alongside can be prepared for algal production as described in the preceding section. By the time the rice seedlings are ready for transplantation, an amount of 15 to 20 kg of algal material will be available and sufficient to inoculate about one and a half hectares. Transplantation is made in the nursery and algal plots and in this way land is neither wasted nor locked up exclusively for algal production during the growing season.

In China, at the Agricultural Research Institute, Nanjing, a mixture of *Anabaena* sp. and *Nostoc* sp. is prepared for field application according to the following procedure (FAO 1977). The algae are first grown in flasks containing sterile medium and are then transferred to large glass bowls under non-sterile conditions. Nursery plots of 5-7 m x 1 m x 20 cm, containing 6-7 cm of water are inoculated at the rate of 150 g algae per m^2. After about seven days the algae attain a biomass of 500 - 1 000 g/m^2. The nursery plots are covered with transparent plastic sheets to protect them from low temperatures (Figure 31). The field inoculation is done by spreading the algae at the rate of 750 kg/ha, which grows to attain a biomass of 7.5 tonnes/ha within 10 - 15 days and if the temperature exceeds 303 kelvin (30 °C) 15 tonnes/ha can be reached.

RECOMMENDATIONS FOR FIELD APPLICATION OF BLUE-GREEN ALGAE

i. If mineral nitrogen fertilizers are not being used, apply blue-green algae in order to gain the benefit of from 20 - 30 kg N/ha.

ii. Broadcast the dry algal material over the standing water in the rice field at a rate of 10 - 15 kg/ha one week after transplanting the seedlings. Addition of excess algal material is not harmful and will accelerate the multiplication and establishment in the field. Providing that the field is not being used and that water facilities are available, algal application can be done well in advance of transplanting the rice.

iii. When mineral nitrogen fertilizer is used reduce its dose by 25 kg/ha and supplement with algal application.

iv. If so desired, algae can also be used along with high levels of nitrogen fertilizer.

v. The sun-dried algal material can be stored for a long time in a dry state without any loss in viability.

vi. Do not store the algal material in direct contact with fertilizers or other chemicals.

vii. Apply algae for at least three consecutive seasons.

viii. Recommended pest control measures and other management practices do not interfere with the establishment and activity of the algae in the field.

ECONOMICS OF ALGAL PRODUCTION AND APPLICATION

The low cost algal technology has an income and employment-generating potential and can be integrated into rural development programmes.

Algal Production

Large-scale algal production is carried out in many experiment stations (Figure 32), State Seed Farms (Figures 33 and 34), village level 'Panchayat Unions' (Figure 35) and by many farmers

At a State Seed Farm in Tamil Nadu, India, where algae are produced using a portion of the threshing floor (8.5 x 7.1 x 0.23 m), the cost of production and the income are as follows:

a. Raw materials

Blue-green algal culture	8.25 kg	US $	2.96
Superphosphate	8.25 kg		0.60
Sawdust	6.50 kg		0.09
Carbofuron	0.81 kg		1.29
Labour charges			1.32
			6.26

b. Produce obtained (algal harvest) 100 kg

c. Cost of harvest production 0.06

d. Return

Cost of material obtained per harvest (100) kg at rate of US $ 0.24 per kg	24.00
In one year of normal conditions the farm can produce 27 harvests; thus total income will be	478.96
Net profit per harvest	17.74

Many farmers use shallow pits layered with plastic sheets or use permanent tanks constructed sometimes on their roof-tops (Figure 37). Based on a village level unit where the farmer employs the pit method (1.5 x 0.3 x 0.2 m), the cost of production works out to about US $ 0.08 per kg of material at the rate of 10 kg per harvest in fifteen to twenty days, sufficient to inoculate one hectare.

Algal Application

Twenty-five kilogrammes of nitrogen in terms of chemical fertilizer will cost around US $ 12.00. To provide this amount of nitrogen through algal inoculation and nitrogen fixation requires about 10 kg per hectare of algal material, which will cost US $ 1.20 to $ 2.40; if the farmer produces his own material, the cost will be less than one dollar.

Table 22 shows the complementation effect of algal application and additional income obtained by the farmers. The average increase in yield is around 10% and the net average additional income is about US $ 59.

Table 22 ADDITIONAL YIELD OF RICE AND INCOME OBTAINED BY FARMERS AS A RESULT OF ALGAL COMPLEMENTATION TO MINERAL FERTILIZATION WITH NPK AT 100:50:50 (District Agricultural Officer, Tenkasi 1978/9)

Location	Variety	Grain yield kg/ha		Percent increase	Additional income US $
		NPK	NPK/algae		
Urkadu	IR20	4 485	4 950	10.3	53.05
Singampetti	IR20	4 960	5 360	8.0	45.51
Brimmadesam	IR20	5 535	6 125	10.6	67.66
Vikramasingapuram	ADT31	5 285	5 935	12.3	75.45
Aventhiruvaleswaram	ADT31	5 045	5 525	9.5	55.00
Kodarankulam	ADT31	4 000	4 935	14.7	73.65
Pappankulam	ADT31	4 065	4 502	10.9	50.90
Kallidaicurichi	ADT31	4 440	4 985	12.2	62.87
Mela Ambasamudram	ADT31	4 340	4 725	11.1	55.69
Keela Ambasamudram	ADT31	4 525	4 995	10.3	53.29
Mean		4 668	5 404	10.9	59.32

5 CONCLUSION

Blue-green algae are one of the major components of the nitrogen-fixing biomass in the paddy fields. The abundance of a sometimes repetitive literature on this subject clearly indicates that researchers have felt the importance and the potentialities of BGA in rice cultivation. Unfortunately despite direct and indirect evidences of their beneficial role the ecology of BGA in rice fields their modes of action on the plant are still imperfectly understood, mainly because of the absence of an easy and accurate technology to study BGA in situ.

Potentialities of BGA depends on environmental conditions. On an average they contribute around 30 kg N/ha in the wetland ecosystem. Some cultural practices like deep-placement of fertilizers can enhance phototrophic nitrogen fixation. Algalization in rice fields has proceeded beyond the stage of fundamental research and attempts have been made to popularize this technology among Indian farmers. BGA, as well as any other biofertilizer, should not be considered as a general recipe for universal application but large scale experiments in India have demonstrated that, if environmental conditions are favorable, BGA inoculation provides an efficient additional source of fertilizer for rice.

6 SOME REFERENCE BOOKS ON BLUE GREEN ALGAE

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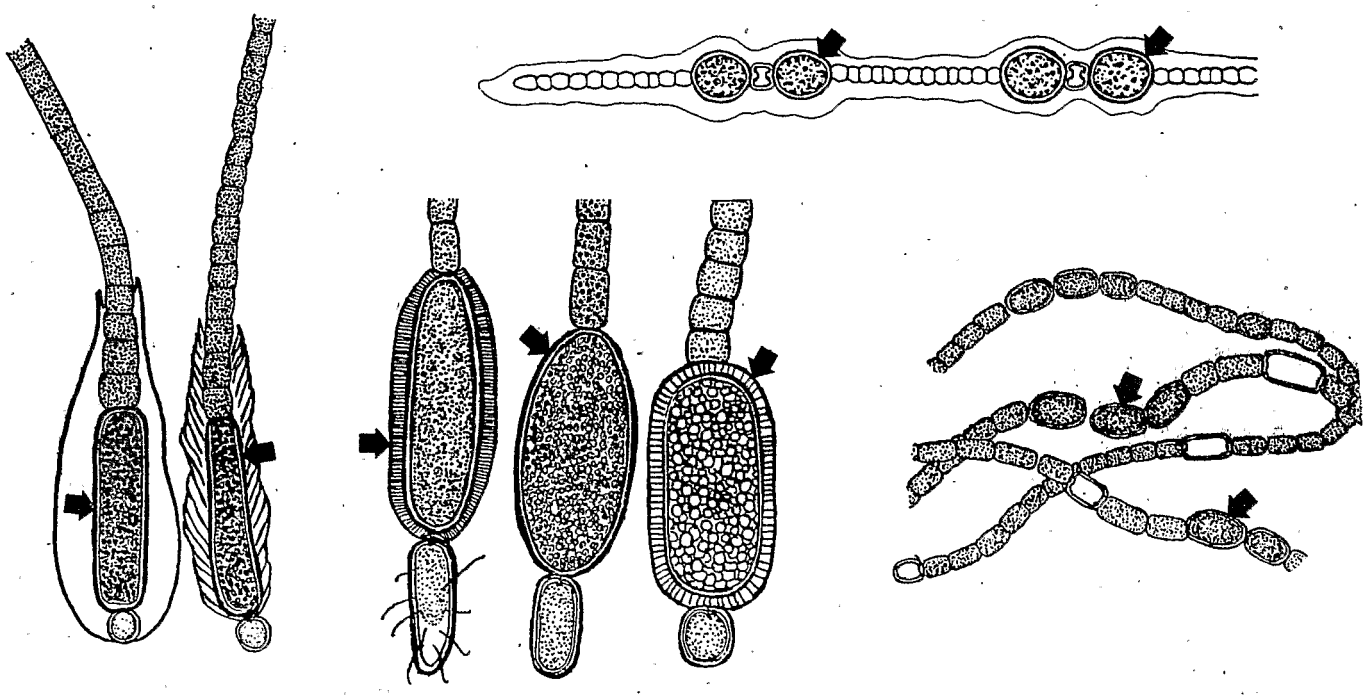
- Roger, P. A. and P. A. Reynaud. Ecology of blue green algae in paddy fields.
- Stewart, W. D. P., P. Rowell, J. K. Ladha and M. J. A. M. Sampaio. Blue-green algae (Cyanobacteria). Some aspects related to their role as sources of fixed nitrogen in paddy soils.
- Venkataraman, G. S. Algal inoculation of rice fields.

A GLOSSARY FOR BLUE-GREEN ALGAE

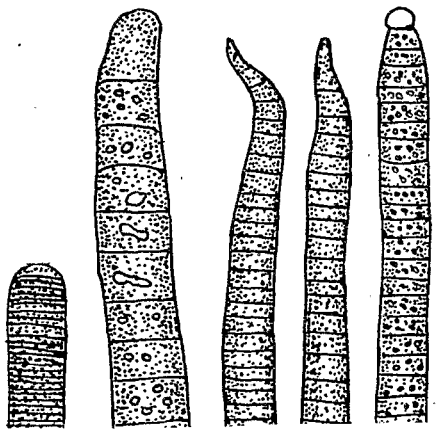
AKINETE: A thick-walled non motile reproductive spore derived from a vegetative cell in which food has been concentrated.

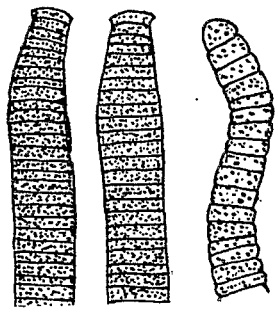


Akinetes from Anabaena (a) Gloeotrichia (b), Cylindrospermum (c), Nostoc (d)

ANTIBIOTIC: A chemical agent or substance produced by an organism which inhibits the growth of another (or itself). Production of antibiotics by BGA has been demonstrated.

APICAL: At the anterior end (apex); apical growth - growth at the apex of a trichome. The shape of the apical cell in sometimes used as a taxonomic character for BGA. Growth of the trichome can be either intercalary or apical depending on the genus.





Different kinds of apical cells in Oscillatoria

APLANOSPORE: Zoospore that has omitted the motile period.

APLANOSPORANGIUM: A cell or sporangium that contains aplanospores.

AUTOTROPHIC: Self-feeding, producing organic matter through photosynthesis. BGA are primarily autotrophic but some of them can grow under heterotrophic conditions.

AUXOTROPHIC: Nutrition in which organic compounds such as vitamins or amino acids are required.

AXENIC: A population of individuals of one strain free from other strains; synonym = pure culture. Obtention of axenic culture of BGA is frequently difficult and tedious. However such culture are absolutely necessary for conducting physiological experiments. In particular the demonstration of the ability of a strain to fix nitrogen has to be done imperatively with an axenic culture.

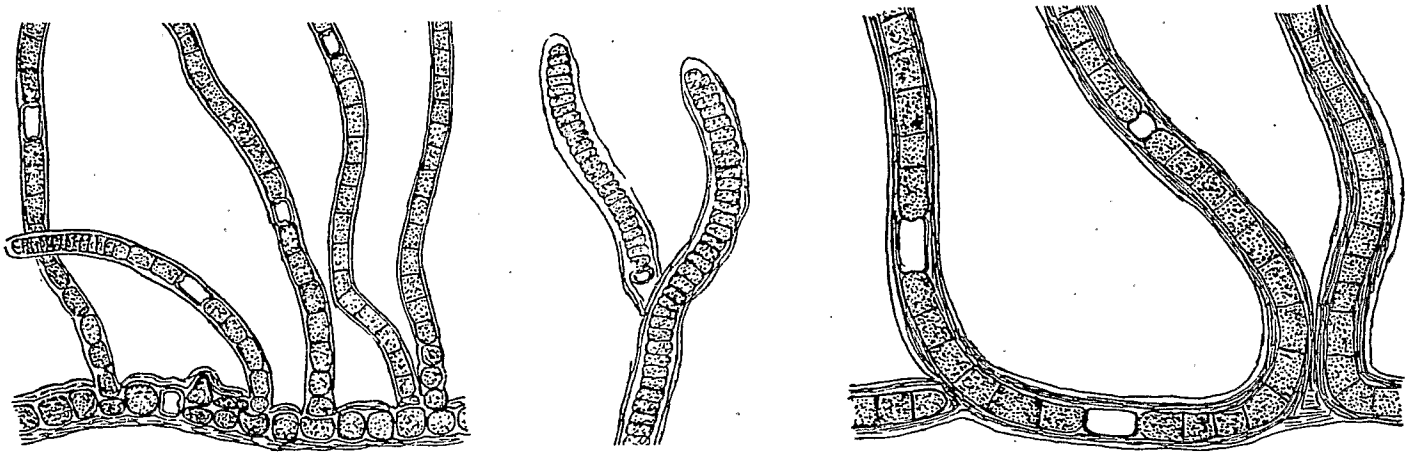
BENTHIC: In an aquatic habitat, attached to a substrate.

BILIPROTEIN: A chromoprotein in which the prosthetic group is a pigment tightly bound by covalent linkages to its apoprotein.

BINARY FISSION: Division into two products. It is the most frequent mode of multiplication in BGA cells.

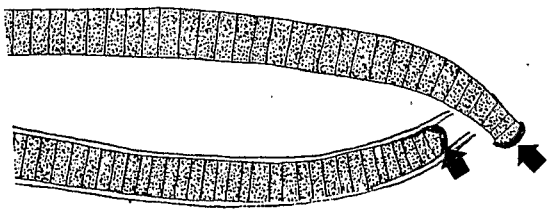
BLOOM: A profuse growth of microscopic or semi-microscopic algae which discolors water; may be of short duration. May also occurs on wet soil. A significant N input in a paddy field can be expected every time a bloom of N_2 fixing BGA develops.

BRANCHING: BGA can develop two kinds of branching. True branching is produced by the vertical division of cells in a main axis as in Fischerella. False branching is the result of the extrusion of the filament throughout the sheath which may produce either a Y false branching as in Tolypothrix or a germinate false branching as in Scytonema.



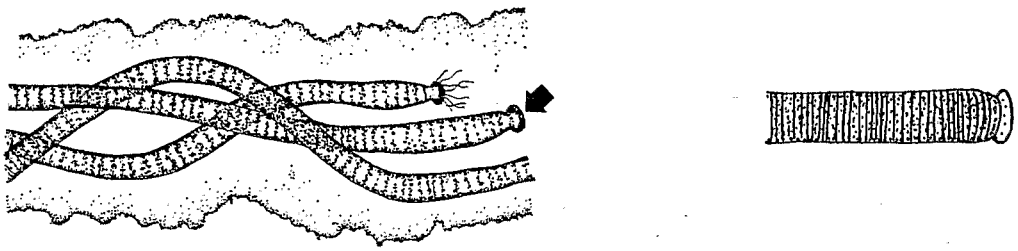
Fischerella Tolypothrix Scytonema

CALYPTRA: A thickening of the cell wall at the apex of a filament, forming a membranous cap; applied to a lid-like covering. The presence or absence of calyptra is a taxonomic character at the species level.



Calyptra in Phormidium uncinatum

CAPITATE: Enlarged or swollen at the apex; with a head. Its a taxonomic character at the species level.



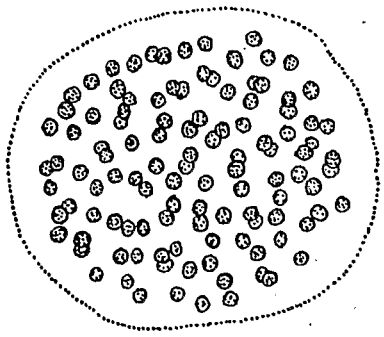
Capitate filaments of Hydrocoleum (a) and Oscillatoria (b)

CENTROPLASM: A central area of the BGA cell (central body) with less pigment than the peripheral. It contains the granules of nuclear material.

CHLOROPLAST: A double-membrane-bounded organelle containing membranous sacs known as thylakoids. It contains chlorophyll a and other components of the photosynthetic reactions. BGA do not have any chloroplasts.

CHLOROPLASM: The peripheral plasm of BGA cell, usually more densely pigmented than the central body.

COCCOID: Round or subsphaerical cells, usually lying free from one another within mucilage, as Gloeocapsa and Aphanocapsa



Colony of Aphanocapsa

COMPENSATION POINT: The level of photosynthesis which just equals or balances respiration. Under a dense rice canopy light intensity may be insufficient and algal photosynthesis may decrease beyond the compensation point.

CYST: A thick-walled dormant cell or stage, usually a metamorphosed vegetative cell in a resting stage; regenerates by forming one or more new organism. The name "heterocyst" was given to thick-walled BGA's cells before knowing their predominant role in nitrogen fixation.

DENDROID: Branching irregularly, similar to that of a root system.

DICHOTOMOUS: Branching in a forking fashion to form two branches.

EDAPHIC: Of the soil.

EUKARYOTIC: Having membrane-bounded nuclei. Contrary: Prokaryotic. BGA are prokaryotic organisms as other algae are eukaryotic organisms.

EUPHOTIC ZONE: The layer of water or soil receiving sufficient light for photosynthesis occur. In submerged paddy fields the euphotic zone generally comprises the submersion water and the few first millimeters of soil. A dense canopy and a muddy water may reduce the depth of the euphotic zone.

EURYHALINE: Having a broad tolerance to varying salinity. Some BGA can tolerate from 35 g/ℓ to 350 g/ℓ NaCℓ.

EUTROPHIC: A high productive water, rich in nutrients. BGA are an index of the level of eutrophication in freshwater ecosystems.

EXOSPORES: Spores of BGA abstricted from the protoplasm of the parental cell.

FILAMENT: For BGA, comprises the trichome (row of closely adjoined cells) and the sheath when there is one.

FRAGMENTATION: Formation of new individuals from segments arising by the breakup of a parental filament. This mode of reproduction is the most frequent among BGA.

ENDO: inside.

Endocytic: living within the cell.

Endolithic: rock-penetrating.

Endophytic: within tissues of a plant.

Endozoic: within tissues of an animal.

All these modes of life are represented within BGA.

ENDOTOXIN: Poisonous substances produced and retained within a cell, and released only after death of the cell (contrary: exotoxin). BGA as Schizothrix calciola and Anabaena flos-aquae produce lipopolysaccharide endotoxins.

ENDOSPORE: A BGA spore formed by multiple internal divisions of the protoplasm of a vegetative cell. Endospore are not enclosed by a thick wall but only by a membrane. Endospore are formed by BGA belonging to Chamaesiphonales.

EPI: on

Epidaphic: living on the soil surface.

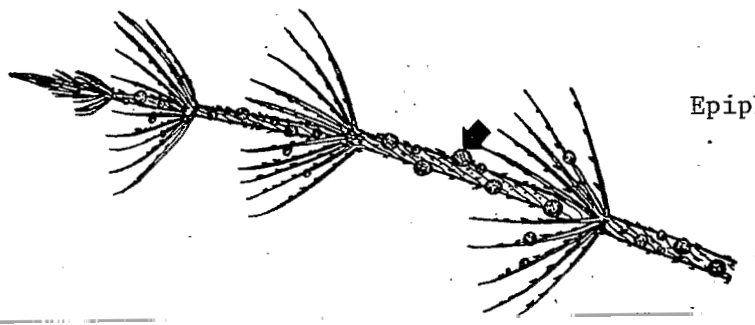
Epilithic: living upon rocks and stones.

Epipelic: attached to mud or sand.

Epiphytic: growing on a plant (sometimes specifically associated).

Epiterranean: subaerial or terrestrial algae forming an association only at the surface of the soil.

Epizoic: living on animals (sometimes specifically associated).



Epiphytic Gloeotrichia on Chara.

GLIDING MOVEMENT: Movement of organism without flagella or pseudopodia when in contact with a substrate. Gliding movement is characteristic of some Oscillatoriales and Nostocales.

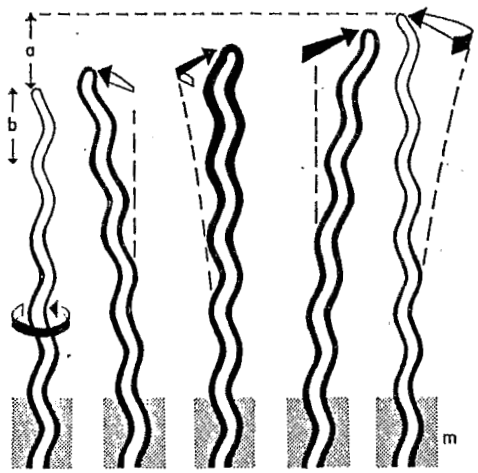
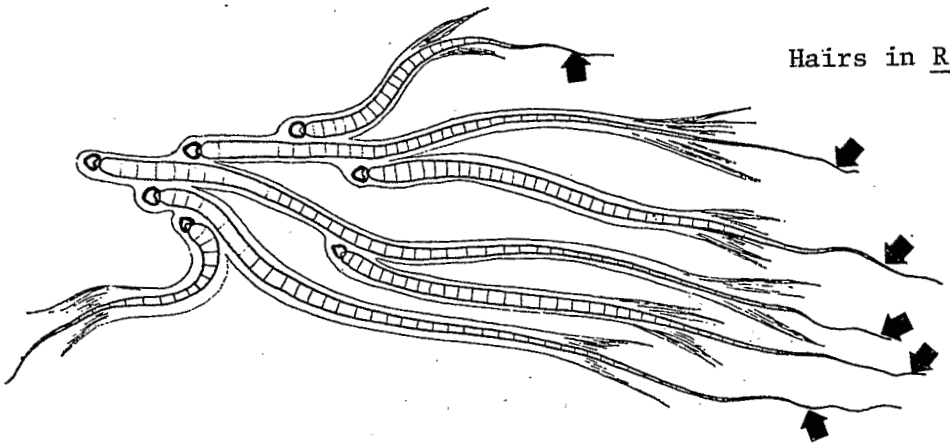


FIG. 6.4. Diagram showing five stages in the progress of a bent filament rotating in the direction of the arrow, as it glides through the mucilage (m). The end of the filament prescribes a conical path, as indicated by the thickness of the lines (thin lines into the page, thick lines out of the page). The wavelength (a) of the oscillations is the same as the distance travelled in one rotation and corresponds to the wavelength of the morphological spiral, where this occurs (b).

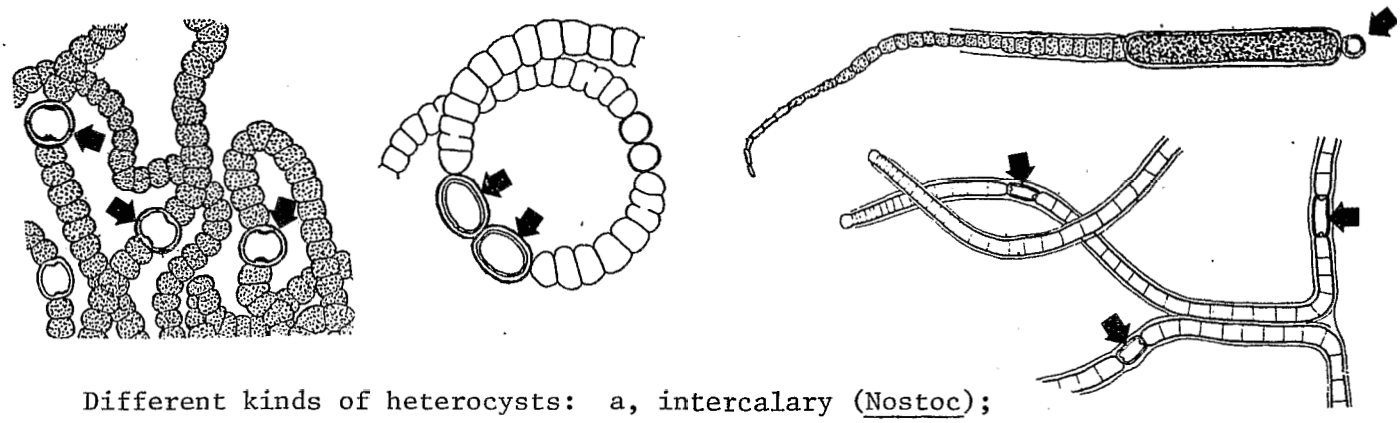
Reproduced from Fogg et al - 1973. "The Blue-Green Algae".

HAIRS: Colorless, typically elongate, unicellular or multicellular structures. In Rivularia and Gloeotrichia the trichome taper and the terminal cells become depleted of cell content so that distinct hair are produced.



Hairs in Rivularia nitida.

HETEROCYST: A thick-walled, usually transluscent cell of certain BGA (heterocystous BGA) known to be the site of nitrogen fixation heterocysts occurs either basally or intercalary.

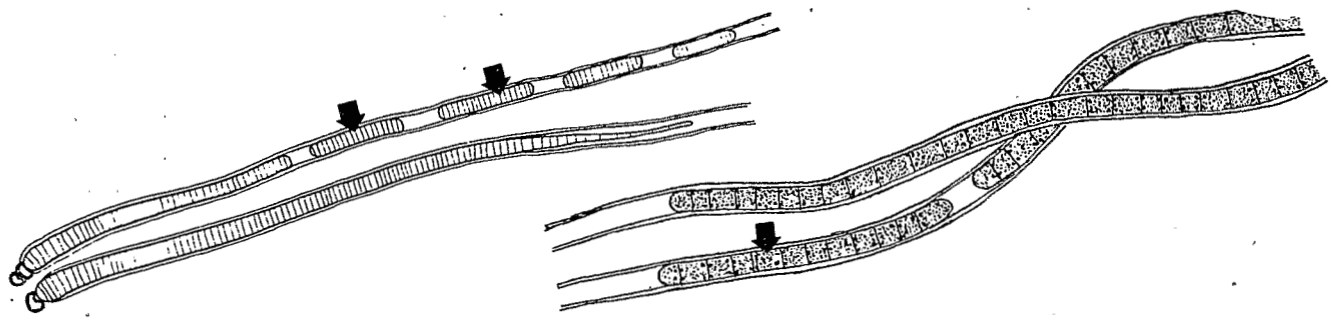


Different kinds of heterocysts: a, intercalary (Nostoc); b, intercalary and geminate (Anabaenopsis): c, intercalary and rectangular (Scytonema): d, terminal (Gloeotrichia).

HETEROTRICHOUS: A thallus which is partly prostrate and partly erect. Example Fischerella.

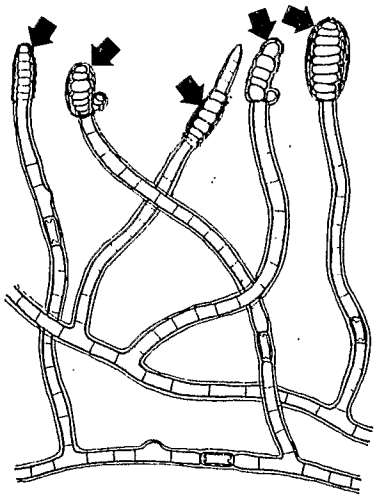
HETEROTROPHIC: Obtaining food from organic substances in the medium. Some BGA are able to grow autotrophically.

HORMOGONIUM: A usually motile segment of a BGA capable of growing into another filament or a short section of a BGA filament.



Hormogonia in a Calothrix filament (a) and in a Lyngbya filament (b)

HORMOSPORE: A spore-like body formed by a short section of a filament becoming invested by a thick membrane and acting as a dormant reproductive element. Synonym hormoscyst. Example Westiella lanosa.



MULTISERIATE: Thallus composed of several series of cells. Example Stigonema.

NECRIDIUM: A dead cell: a cell in the trichome of the Oscillatoriales which dries, becomes filled with mucilage, and so forms a weak link, providing for fragmentation.

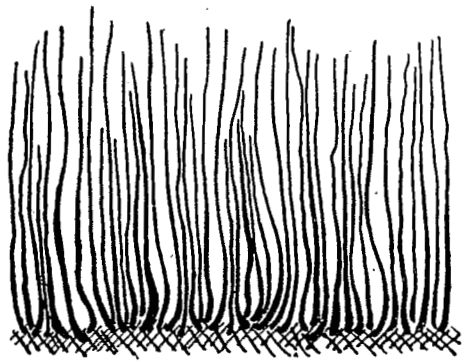
NITROGEN FIXATION: Incorporation of atmospheric nitrogen as a nitrogen source into the cells of certain BGA and bacteria, by the reduction of dinitrogen to ammonia.

OLIGOTROPHIC: Water with few electrolytes and with other features which render it low in productivity of biota-oligotrophic waters have less than 100 ppm of solution.

PALMELLOID: A thallus involving an indefinite arrangement of many (usually spherical) cells embedded in mucilage.

PARENCHYMATOUS: A thallus composed of a cushion-like mass of cells all about the same size and shape.

PENICILLATE: Brush-like



Penicillate thallus of Calothrix

PERIPHYTON: Association of organism attached to and/or growing over submerged plants.

PHOTOAUXOTROPHIC: Photosynthetic but requiring vitamins or amino acids.

PHOTOTACTIC: Moving in reaction to light of various intensities.

PHOTOTROPHIC: Moving in the direction of light source.

PHYCOBILIN: Biliprotein pigments of BGA and red algae.

PHYCOBILISOME: The cellular organelle on the surface of thylakoids in which the biliprotein pigments are presents in BGA and red algae.

PHYCOCYANIN: Blue biliprotein pigment of BGA and red algae.

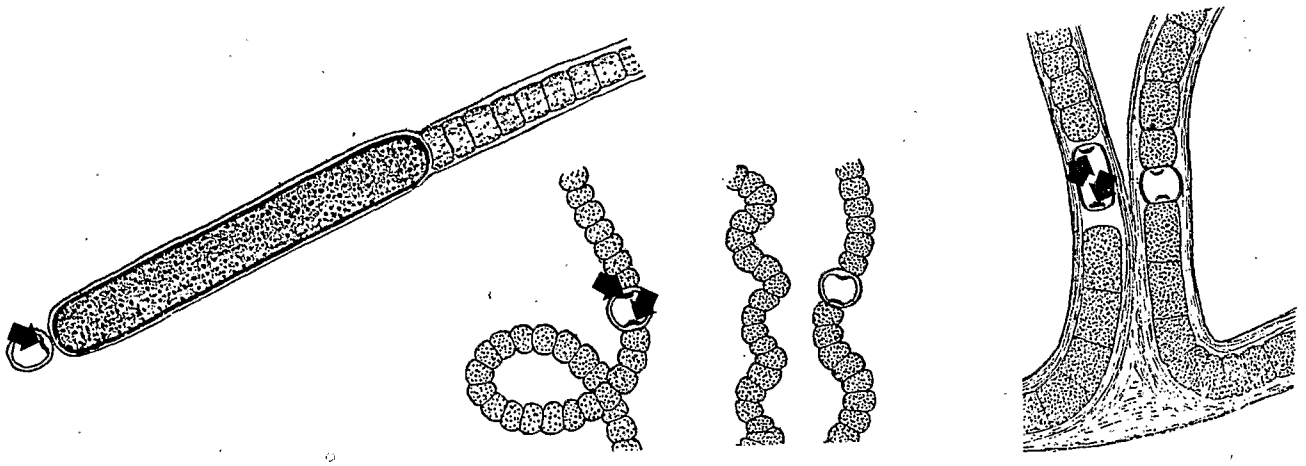
PHYCOERYTHRIN: Red biliprotein pigment of BGA and red algae.

PHYCOVIRUS: Virus causing lysis of algal cells. Cyanophages, specific of BGA, are phycovirus.

PLANKTON: The community of small organisms suspended in water.

PLASMODESMA: Delicate protoplasmic connections between cells arising by incomplete cytokinesis.

POLAR NODULE: Thickenings in the heterocyst walls at the front of attachment of heterocysts to adjacent cells.



a: terminal heterocyst of Gloeotrichia with one polar nodule.

b and c: intercalary heterocysts of Nostoc (b) and Scytonema (c) with two polar nodules.

PROKARYOTIC: Lacking membrane-bounded DNA (and Golgi apparatus, mitrochondria, and plastids). BGA are prokaryotic microorganisms.

PROPAGULE: A multicellular structure functioning for asexual reproduction.

PSEUDOVACUOLE: A gas-filled packed in cells of many BGA, usually light refractive.

PURE CULTURE: See AXENIC

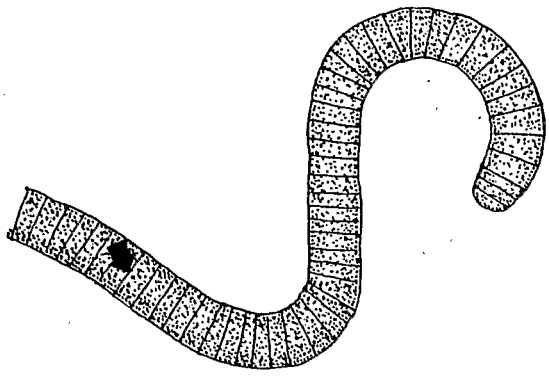
REPRODUCTION:

Asexual: increase in number of individuals not involving gametic union.

Sexual: increase in number of individuals involving union of gametes. Sexual reproduction has not yet been observed for BGA.

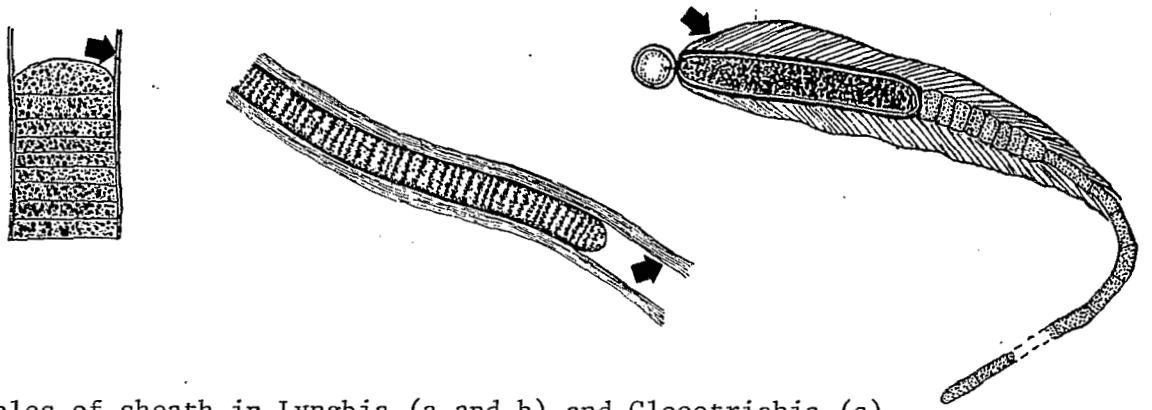
SAXICOLOUS: Growing on rocks or rocky substrata.

SEPTA: Cross partition or walls usually complete, sometimes interpreted



Example of septa in Arthrospira platensis.

SHEATH: An (often pectinaceous) investment outside the wall of certain algal cells.



Examples of sheath in Lyngbia (a and b) and Gloeotrichia (c).

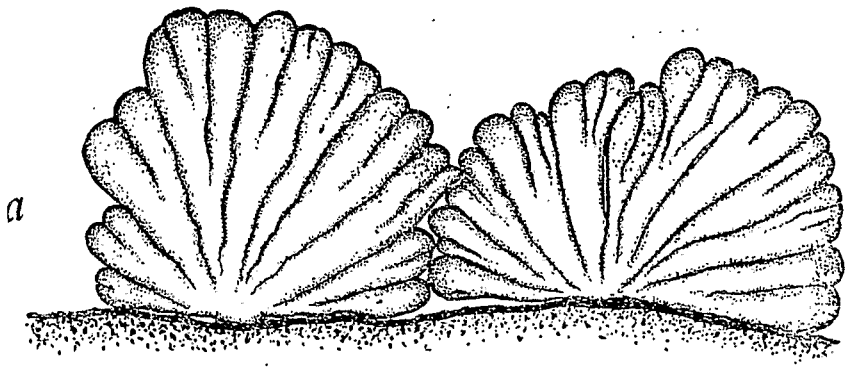
SPORE: A cellular agent of asexual reproduction. (in BGA synonym: akinete).

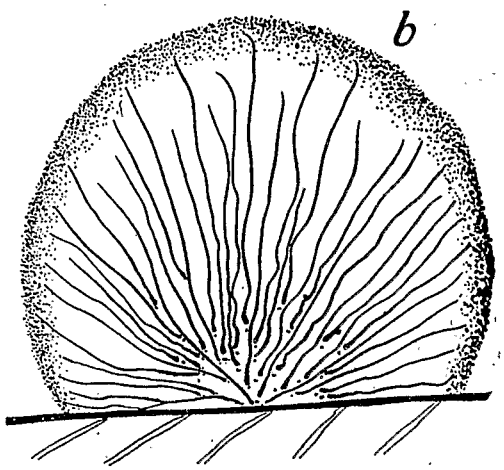
STROMATOLITE: A fossilized, calcareous aggregate of BGA.

SYMBIOTIC: Symbiotism, two or more organisms living together or in close association, with various degrees of interdependence or parasitism.

TAXON: A recognized, systematic entity of whatever ranks.

THALLUS: A plant body not differentiated into vascularized roots, stem, and leaves.

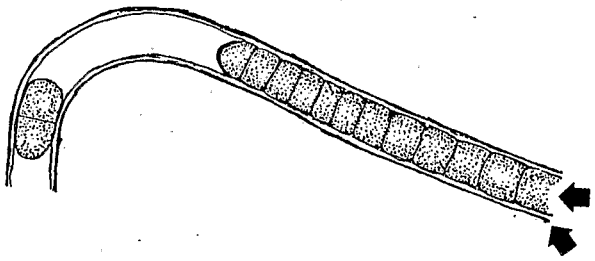




Thalli of Nostoc letestui (a) (after Fremy) (x 4)) and Rivularia dura (b) (after Fremy) (x20)).

THYLAKOID: A photosynthetic lamella or sac.

TRICHOME: A hair of gelatinous bristle, or an extension of the cell wall; name applied to the thread of cells in filamentous BGA, minus the sheath.



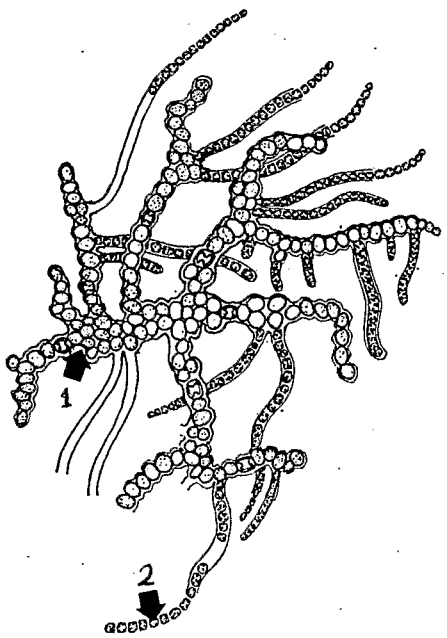
Trichome + Sheath = Filament

UMBROPHILIC: Shade loving, referring to subdued light.

UNIALGAL: A culture containing only one strain or species of alga - does not implies that it is a pure culture.

UNIAXIAL: Having an axis composed of a single filament.

UNISERIATE: A filament in which there is a single series of cells, as opposed to a filament with more than one series.



Fischerella musicola (after Fremy (x 200)) exhibiting both multiseriate (1) and uniseriate (2) filaments.

WATER BLOOM: A concentrated population of planktonic algae microscopically apparent.